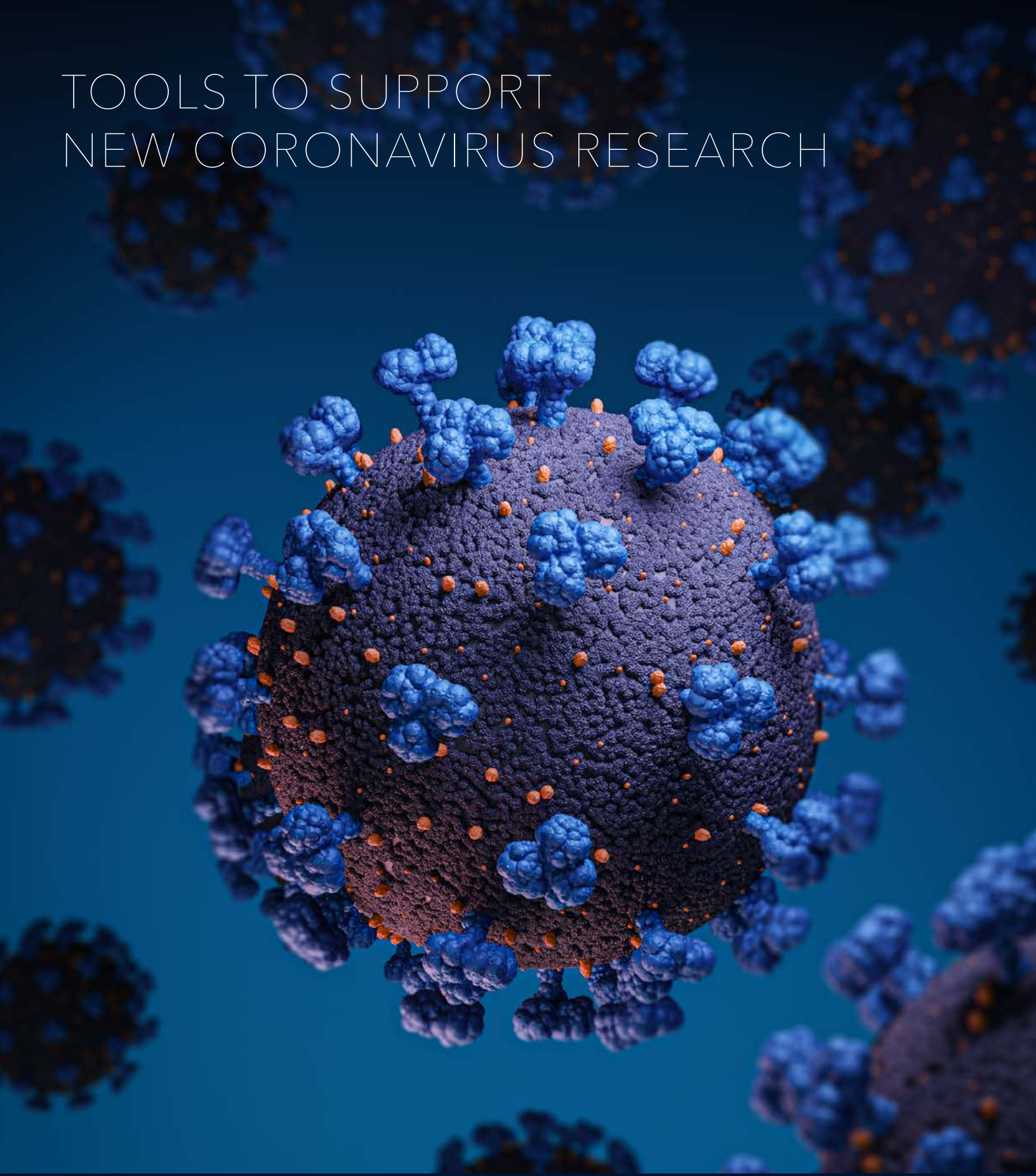
TOOLS TO SUPPORT NEW CORONAVIRUS RESEARCH



bio-techne®

On February 11, 2020, the new coronavirus (CoV) discovered in Wuhan was officially renamed SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV). It was determined that the virus is genetically related to SARS-like coronaviruses and a strain of the same genus. Coronaviruses are a class of RNA viruses widely found in birds and mammals, including humans. Prior to the emergence of SARS-CoV-2, six coronaviruses had been found to cause human infection. Four strains of coronavirus in circulation globally (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-KHKU1) are responsible for one-third of "common cold" infections. The other two are zoonotic coronaviruses implicated in the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreaks in 2003 and 2012, respectively. Sars-CoV-2 causes a potentially fatal atypical pneumonia, named Coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). The emergence of this new coronavirus (SARS-CoV-2) will surely be engrained in everyone's memory as the infection continues its course worldwide.

Bio-Techne supports research on the detection and prevention of infectious viruses. As a leading company in the life sciences research sector, Bio-Techne develops resources to support coronavirus research, including tools for SARS-CoV-2 detection, cytokine monitoring, and drug discovery, helping the scientific community understand the mechanisms of infection and develop effective treatments.

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MEASURE THE INFLAMMATORY RESPONSE DURING COVID-19 IN REAL TIME WITH ELLA

ON THE FRONT LINE OF FIGHTING COVID-19?
WE'RE HERE TO HELP!

SEE PAGE 14 TO LEARN MORE

SARS-COV-2 DIAGNOSIS

Currently, the diagnosis of SARS-CoV-2 infection recommended by the WHO depends on the comprehensive analysis and detection of viral genes, such as the envelope (*E*) and the RNA-dependent RNA polymerase genes, from clinical samples by reverse transcription polymerase chain reaction (RT-PCR). Alternatively, the US Centers for Disease Control and Prevention recommends the detection and analysis of the *N* gene, which encodes a viral nucleocapsid phosphoprotein. Additional diagnostic testing involves serological detection of IgM/IgG antibodies to viral proteins and viral isolation from clinical specimens. Lastly, epidemiological history is part of the clinical evaluation and helps define specific risk factors for infection. Clinical symptoms are mainly fever, dry cough, and shortness of breath, with pneumonia seen in more severe cases. Chest CT scans are used to complement RT-PCR analysis and often reveal pulmonary bilateral ground-glass opacification and infiltrates. Pulmonary consolidation may occur in severe cases. Other indicators evaluated include total number of white blood cells as well as lymph cell and lactate dehydrogenase (LDH) levels. Severe patients often have elevated inflammatory cytokines such as interleukins IL-2, IL-7, and IL-10, granulocyte colony-stimulating factor (G-CSF), and CXCL10/IP-10.

Pulmonary pathological features commonly associated with SARS-CoV-2 include bilateral diffuse alveolar damage, hyaline membrane formation and interstitial mononuclear inflammatory infiltration, as well as unilateral desquamation of pneumocytes and edema. Less frequent findings include multinucleated giant pneumocytes having irregular nuclei distribution, and giant atypical pneumocytes with prominent eosinophilic nucleoli. Flow cytometric analysis of peripheral blood has shown reduced $CD4^+$ and $CD8^+$ T cells that are hyperactivated. In addition, within the $CD4^+$ T cell subset, the frequency of highly proinflammatory $CCR4^+$ and $CCR6^+$ Th17 cells is increased. Severe immune lung damage has been attributed to the increased frequency of proinflammatory Th17 cells and overactive cytotoxic $CD8^+$ T cells.

SARS-COV-2 TRANSMISSION

SARS-CoV-2 is highly infectious. According to preliminary research, R_0 (basic reproduction number) value is 2.2-3.6, which is similar to that of SARS-CoV (3.0) but greater than MERS-CoV (0.8). The fatality rate for SARS-CoV-2 is about 2-3%, which is considerably lower than the fatality rate for both SARS-CoV (~9.5%) and MERS-CoV (~35%) infections. As of March 30, the WHO has reported 741,030 cases of SARS-CoV-2 infections worldwide. Several factors have contributed to its worldwide expansion such as infectiousness level, population movement patterns, and potential asymptomatic transmission. This new SARS-CoV-2 outbreak represents the third instance within the last two decades of the emergence of a highly infectious global threat. SARS-CoV-2 once again has re-focused the public's attention towards the detection and control of infectious viruses.

SARS-COV-2 DETECTION METHODS

Molecular approaches for the detection of SARS-CoV-2 focus on the analysis of the viral genome and serological analysis of antiviral antibodies. Nucleic acid analysis aims to detect virus-specific genes. Real-time quantitative RT-PCR is the method of choice for SARS-CoV-2 detection because of its high specificity and sensitivity.

Serological analysis detects antibody responses initiated by SARS-CoV-2 infection. Because SARS-CoV-2 is a new human infectious agent, strain specific antibodies are not commonly present in the population. During an immune response, IgM antibodies appear in the early stage, followed by the emergence of IgG during the mid to late disease stages. Recently, an assay that detects IgM and IgG antibodies specific for the nucleocapsid protein from SARS-CoV Rp3 has been developed. In contrast to nucleic acid analysis, serological testing is faster and easier, and offers the advantage of allowing evaluation of convalescent patients. However, a potential key disadvantage to these assays is that antibodies may be undetectable during the early stages of viral infection. Additionally, depending on the antigen used, cross-reactivity, or false positive results, may occur due to the presence of antibodies against closely related CoV strains. These immunological assays are often used to complement nucleic acid analysis for confirming diagnoses of patients with negative nucleic acid findings but clinically suspect. Serological assays are also commonly used for research and surveillance of infectious diseases.

SEROLOGY TEST FOR SARS-COV-2			
PRODUCT	BRAND	CATALOG #	DESCRIPTION
COVID-19 IgG/IgM Rapid Test Kit	Novus Biologicals	NBP2-89106	Chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies against SARS-CoV-2 in human whole blood, serum, or plasma.

SECONDARY ANTIBODIES FOR DEVELOPING IgG AND IgM ELLISAS							
MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE	HOST
IgG Fc	Novus Biologicals	NB7446	Human	Poly	ELISA, EM, ICC/IF, IHC, WB	Yes	Goat
IgG Fc	R&D Systems	MAB110	Human	97924	CyTOF, Flow, WB	Yes	Mouse
IgG Fc	R&D Systems	G-102-C	Human	Poly		No	Goat
IgG	Novus Biologicals	NBP1-51523	Human	4D2D9G8	ELISA, WB	No	Mouse
IgG	R&D Systems	MAB11012*	Human	1268C	ELISA, Flow	Yes	Rabbit
IgG	R&D Systems	MAB11013*	Human	1268A	ELISA	No	Rabbit
IgG	R&D Systems	F0135	Human	Poly	Flow	Yes	Goat
IgG	R&D Systems	G-101-C-ABS	Human	Poly		No	Goat
IgM F(ab')2	Novus Biologicals	NBP1-75024	Human	Poly	CLIA, ELISA, Flow, IA, ICC/IF, IHC, IM, WB	Yes	Goat
IgM Fc fragment	Novus Biologicals	NB500-468	Human	CH2	ELISA, Flow, ICC/IF, WB	Yes	Mouse
IgM µ Chain	R&D Systems	G-105-C	Human	Poly		No	Goat
IgM	Novus Biologicals	NBP1-75077	Human	Poly	CLIA, ELISA, Flow, IA, ICC/IF, IHC, IM, WB	Yes	Rabbit
IgM	Novus Biologicals	NBP2-60670	Human	Poly	ELISA, FLISA, ICC/IF, IHC, WB	Yes	Goat
IgM	R&D Systems	MAB9435*	Human	2123A	ELISA, SW, WB	No	Rabbit
IgM	R&D Systems	MAB94351*	Human	2123C	ELISA, SW, WB	No	Rabbit

Species Key: + Additional Species Available

Applications Key: CLIA Chemiluminescence Immunoassay, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **EM** Electron Microscopy, **FLISA** Fluorophore-linked Immunosorbent Assay, **Flow** Flow Cytometry, **IA** Immunoassay, **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **IM** Immunomicroscopy, **SW** Simple Western™, **WB** Western blot

*Indicates a recombinant monoclonal antibody

Learn more | novusbio.com/support/generalsecondaryantibodies

EXOSOME ANALYSIS WITH EXOSOME DX™

Exosome Diagnostics (ExosomeDx™) is the clinical diagnostic laboratory service of Bio-Techne. It is focused on the development and commercialization of revolutionary biofluid-based diagnostics to deliver personalized precision healthcare that improves lives using exosome analysis. Exosomes are small lipid vesicles that are very similar in structure to enveloped viruses like the coronavirus. ExosomeDx has clinical laboratories for high complexity testing in both the United States (Waltham, MA) and Europe (Munich, Germany). The Waltham CLIA lab is New York state-certified, ISO15189, and has a BSL-2 environment devoted to performing diagnostic testing from a range of different biofluids.

The ExosomeDx platform can yield comprehensive and dynamic molecular insights to transform how diseases are diagnosed, treated, and monitored. To aid with this SARS-CoV2 crisis, we have pivoted resources to help solve the current lack of diagnostic testing. We are available to partner with major health care systems, researchers, and providers to leverage our expertise by developing and performing assays such as:

- SARS-CoV-2 diagnostic qPCR testing
- SARS-CoV-2 serology assays
- Novel RNA biomarkers in plasma to monitor drug treatment and disease-associated responses with complete RNA profiling

The expertise in exosome isolation and biomarker development has made ExosomeDx the "go-to" biomarker platform for many pharmaceutical companies that utilize these capabilities to diagnose, stratify, and monitor patient response in various clinical studies. The proprietary exosome isolation platform enables complete RNA (monitor thousands of targets simultaneously) and protein markers (cell surface and encapsulated) profiling. Exosomes also play a role in normal physiology and monitoring them in biofluids can give information on many different pathways and processes (see the following citations: García-Silva, S. *et al.*, 2019; Hydbring, P. *et al.*, 2018; Krug, A.K. *et al.*, 2018; McKiernan, J. *et al.*, 2018; Simonson, O.E. *et al.*, 2016; Zanello, S.B. *et al.*, 2018). Exosomes have generated significant diagnostic and therapeutic opportunities, including most recently, a clinical trial using therapeutic exosomes for severe COVID-19 (NCT04276987).

Learn more | exosomedx.com

RNAscope™ *IN SITU* HYBRIDIZATION ASSAYS

RNAscope™ assays enable researchers to study SARS-CoV-2 infections in host tissues by chromogenic and fluorescent *in situ* hybridization (ISH). With over 400 publications, researchers worldwide have successfully applied this cutting-edge technology in pursuit of a better understanding of DNA viruses, RNA viruses, and retroviruses. In particular, this molecular virology tool has been used to accurately pinpoint the site and spread of the viral infections in different tissues and organs, as well as in developing and verifying animal models, determining the pathogen-elicited immune response, and for evaluating the efficacy of vaccines. For a multi-omics approach, combining RNAscope™ ISH with immunohistochemistry (IHC) allows spatially resolved gene expression analysis at the RNA and protein level to understand complex multicellular interactions within the tissue.

RNAscope™ uses Advanced Cell Diagnostics' (ACD) unique, patented probe design strategy to enable simultaneous signal amplification and background noise suppression. Unlike traditional ISH methods, its specific double-Z probe design prevents amplification of non-specific signals. In addition, the three double Z probe design and signal amplification increases sensitivity such that even a single molecule of RNA can be detected.

Recently, ACD designed RNAscope™ probes from the SARS-CoV-2 genome. Using the RNAscope™ detection kit and accompanying protocol, SARS-CoV-2 can be detected and visualized in various tissues/organs, similar to studies investigating MERS-CoV (Figure 1). In addition to selecting a predesigned probe for SARS-CoV-2, researchers also have the option to design made-to-order target probes for examining different regions of interest.

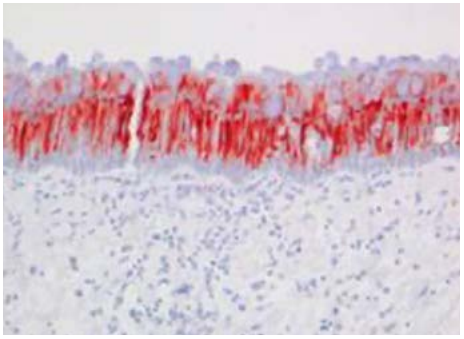


Figure 1. Middle East respiratory syndrome coronavirus (MERS-CoV) is another pathogenic coronavirus. During the development of the viral vaccine, Haagman *et al.* demonstrated the efficacy of the vaccine using RNAscope™ technology. MERS-CoV viral RNA was detected in the nasal respiratory epithelium of camels using an RNAscope probe that specifically targets the nucleocapsid mRNA of the MERS-CoV virus. (positive ISH signal is shown in red). To learn more about this study please refer to Haagmans, B.L. *et al.* (2016) Science **351**:77 (doi: 10.1126/science.aad1283).

Image Courtesy of Bart L. Haagmans.

RNAscope FOR SARS-COV-2		
CATALOG #	PROBE NAME	DESCRIPTION
848561	RNAscope Probe - V-nCoV2019-S	Designed to specifically detect the Spike (S) protein of the Novel Coronavirus to avoid cross detection of SARS-CoV, MERS-CoV, other coronaviruses, Ebola virus or HIV.
845701	RNAscope Probe - V-nCoV2019-S-sense	A "sense" probe designed to target the antisense strand of the S gene, which can be used to visualize viral replication in individual cells.
848151	RNAscope Probe - Hs-ACE-2	Provides highly sensitive detection of ACE-2 receptor expression in cells and tissue sections.
470341	RNAscope Probe - Hs-TMPRSS2	Detects the transmembrane serine protease TMPRSS2.
310361	RNAscope Probe – Hs-IL-1β/IL-1F2	Detects the proinflammatory cytokine IL-1β/IL-1F2.
310371	RNAscope Probe – Hs-IL-6	Detects the proinflammatory cytokine IL-6.
310381	RNAscope Probe – Hs-IL-8/CXCL8	Detects the proinflammatory cytokine IL-8/CXCL8.
310421	RNAscope Probe – Hs-TNF-α	Detects the proinflammatory cytokine TNF-α.

Learn more | acdbio.com/science/applications/research-areas/infectious-diseases

CELL CULTURE FOR VIRUS RESEARCH

Viruses are pathogenic intracellular parasites that require living cells to multiply. Animal viruses, like SARS-CoV-2, need cells from a host animal for replication. Cultured host cells provide a convenient and cost-effective method for cultivating animal viruses. Cell culture systems allow for the investigation of viral replication and host-pathogen interaction, virus detection and identification, and vaccine production.

A successful cell culture depends on creating and maintaining optimal culture conditions. Bio-Techne supports cell culture with high quality reagents including cell culture media, serum, defined media supplements, highly bioactive growth factors and small molecules, hydrogels, extracellular matrices, and basement membrane extracts for cell culture attachment. We also offer a wide variety of fetal bovine serum (FBS), manufactured at our ISO 9001:2105 certified facility, to support general cell culture, stem cell cultures, and many other specialized cell culture needs. We also have USDA APHIS-certified, US Origin, and USDA-approved grades of FBS. We understand the vital impact quality FBS has on today's evolving cell culture technology and have, thus, established a network of raw materials to ensure direct control over all processes involved in the manufacture of our FBS.

CELL CULTURE MEDIA

PRODUCT	BRAND	CATALOG #
DMEM, high glucose, no glutamine, no sodium pyruvate	R&D Systems	M22850
RPMI 1640, GlutaminePlus	R&D Systems	M30850
RPMI 1640, L-glutamine	R&D Systems	M30150

ANTIBIOTICS/ANTIFUNGALS

PRODUCT	BRAND	CATALOG #
Gentamicin (10 mg/mL)	R&D Systems	B20192
Penicillin-Streptomycin 10/10 (100X)	R&D Systems	B21210
Penicillin-Streptomycin 5/5 (100X)	R&D Systems	B21110
Antibiotic-Antimycotic (100X)	R&D Systems	B22110
Amphotericin B (Fungizone)	R&D Systems	B23192

FETAL BOVINE SERUM

			CATALOG #S	
PRODUCT	BRAND	VOLUME	REGULAR	HEAT INACTIVATED
Fetal Bovine Serum – Optima	R&D Systems	50 mL	S12495	S12495H
Fetal Bovine Serum - Optima	R&D Systems	100 mL	S12410	S12410H
Fetal Bovine Serum - Optima	R&D Systems	500 mL	S12450	S12450H
Fetal Bovine Serum - Premium	R&D Systems	50 mL	S11195	S11195H
Fetal Bovine Serum - Premium	R&D Systems	100 mL	S11110	S11110H
Fetal Bovine Serum - Premium	R&D Systems	500 mL	S11150	S11150H
Fetal Bovine Serum – Premium Select	R&D Systems	50 mL	S11595	S11595H
Fetal Bovine Serum - Premium Select	R&D Systems	100 mL	S11510	S11510H
Fetal Bovine Serum - Premium Select	R&D Systems	500 mL	S11550	S11550H

BUFFERS, SOLUTIONS AND ADDITIONAL REAGENTS

PRODUCT	BRAND	CATALOG #
Cell Freezing Medium – II	R&D Systems	B71295
DPBS, no Ca^{2+} and Mg^{2+} salts, no phenol red	R&D Systems	B30250
Fico/Lite-LM (Mouse)	R&D Systems	I40650
Fico/Lite-LymphoH™	R&D Systems	I40150
GlutaminePlus – 200mM (100X)	R&D Systems	B90210
GlutaminePlus, powder	R&D Systems	R90210
L-Glutamine - 200mM (100X)	R&D Systems	B90010

BUFFERS, SOLUTIONS AND ADDITIONAL REAGENTS

PRODUCT	BRAND	CATALOG #
L-Glutamine, powder	R&D Systems	R90010
HEPES (Ultra Pure), powder	R&D Systems	R35150
HEPES Buffer Solution (1 M)	R&D Systems	B35110
MEM Sodium Pyruvate Solution - 100mM	R&D Systems	B84010
Trypsin 0.05% – EDTA 0.53 mM (1X)	R&D Systems	B81110
Trypsin 0.25% - EDTA 1mM (1X)	R&D Systems	B81350
Trypsin 0.5% - EDTA 5.3 mM (10X)	R&D Systems	B81210
Trypsin 2.5% (10X)	R&D Systems	B81710

Learn more | rndsystems.com/cellculture

THERAPEUTICS RESEARCH AND DEVELOPMENT – DEVELOPING ANTI-COVID-19 DRUGS

Each day the number of confirmed SARS-CoV-2 infections worldwide is rising, creating an urgent need to develop effective treatments. At present, researchers are focused on three key areas for potential treatments: specific antiviral drugs, stem cell therapies, and vaccine development. Drug development for COVID-19 can be classified into four categories:

I. Testing new uses for existing antiviral drugs
II. Screening small molecule libraries
III. Developing antiviral drugs that target specific players in the SARS-CoV-2 life cycle
IV. Monitoring disease progression and targeting the cytokine storm

TESTING NEW USES FOR EXISTING ANTIVIRAL DRUGS

Developing new drugs that are safe and effective is both time-consuming and costly. However, repurposing existing drugs, also known as drug repositioning, is a strategy that may enable a more rapid response to infectious disease outbreaks. Since the efficacy and safety of FDA approved drugs have been established, the amount of time required to bring a repurposed drug to the market may be dramatically reduced.

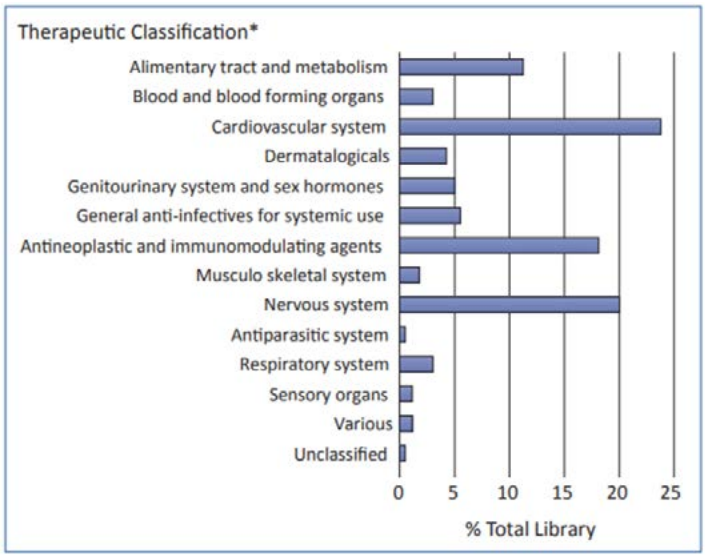


Figure 2. The Tocriscreen Library of FDA-Approved Drugs (Tocris, Catalog # 7200, coming soon) offers 190 FDA-approved compounds supplied pre-dissolved in DMSO. This library of compounds is ideal for screening assays for drug repurposing.

The first step in finding an effective treatment for SARS-CoV-2 is testing existing antiviral drugs developed against SARS, MERS, Ebola, and HIV. For example, the anti-Ebola drug, Remdesivir, which was developed by Gilead Science Inc. and reported positive results after completion of Phase III clinical trials in the United States, is now being investigated as a potential anti-COVID-19 therapeutic by the NIH. In the fight against COVID-19 infection, many clinical studies have also been initiated in China. Kelizhi, marketed as an anti-HIV therapy in the United States, has recently been registered for a clinical trial in combination with interferons in China. Arbidol, which was approved to treat the flu in China, is also being evaluated. Bio-Techne supports the ongoing research to quickly identify a COVID-19 drug by offering a Tocriscreen compound library of 190 FDA-approved drugs that cover over 12 different therapeutic classifications (Figure 2).

SCREENING SMALL MOLECULE LIBRARIES

Libraries of bioactive compounds can also be screened with the aim of developing a novel therapeutic against COVID-19. Tocris offers a library of 1280 fully annotated compounds for screening and identifying compounds that could target SARS-CoV-2. The Tocriscreen 2.0 compound library, which has very little overlap with other libraries on the market, is available in 3 formats.

TOCRISCREEN COMPOUND LIBRARIES			
	2.0 MICRO (CATALOG # 7152)	2.0 MINI (CATALOG # 7151)	2.0 MAX (CATALOG # 7150)
#. of Compounds	1280	1280	1280
Volume	15 µL	50 µL	250 µL
Solution Format	10 mM DMSO	10 mM DMSO	10 mM DMSO
Seal	Peelable foil seal	SepraSeal Cap	SepraSeal Cap
Storage Format	96-well, v-bottom microplate	96-well racks with Matrix™ storage tubes	96-well racks with Matrix storage tubes
Storage Temperature	-20°C	-20°C	-20°C
Stability (for at least)	6 months, prior to opening	6 months	6 months

Learn more | tocris.com/product-type/tocriscreen-compound-libraries-and-toolboxes

DEVELOPING ANTIVIRAL DRUGS THAT TARGET SPECIFIC PLAYERS IN THE SARS-COV-2 LIFE CYCLE

SARS-CoV-2 and SARS-CoV are homologous, with their RNA sequences sharing ~80% identity. Research on SARS-host protein interactions with both viruses have shown that the viral spike (S) protein binds human ACE-2 located on the surface of mucosal cells, resulting in fusion of viral and cell membranes for viral entry. Viral entry also requires priming of the S protein by host cell proteases including TMPRSS2 and Cathepsin B/L. Involvement of ADAM17/TACE in this process, which cleaves ACE-2, remains questionable.

Since the S protein, ACE-2, TMPRSS2, and Cathepsin B/L, have important functions in the SARS-CoV-2 life cycle, these proteins serve as potential targets for drug development. A recent study by Hoffmann *et al.* demonstrated that ACE-2 and TMPRSS2 are required for host cell entry. Blocking ACE-2 with an Anti-Human ACE-2 Antibody (R&D Systems, Catalog # AF933) and inhibiting TMPRSS2 with the protease inhibitor Camostat attenuated viral entry. This publication also showed that E 64d (Tocris, Catalog # 4545), a cathepsin inhibitor, prevents viral cell entry *in vitro*, in combination with Camostat. Bio-Techne offers various reagents (Figures 3-8) to support the development of neutralizing antibodies and inhibitors against proteins involved in key steps in SARS-CoV-2 infection.

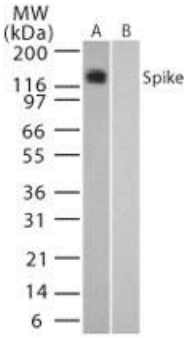


Figure 3. Western blot shows lysates of a mouse melanoma cell line either transfected (**A**) or not transfected (**B**) with a plasmid to express the S protein. The membrane was probed with a Rabbit Anti-SARS Spike Protein Polyclonal Antibody (Novus Biologicals, Catalog # NB100-56578).

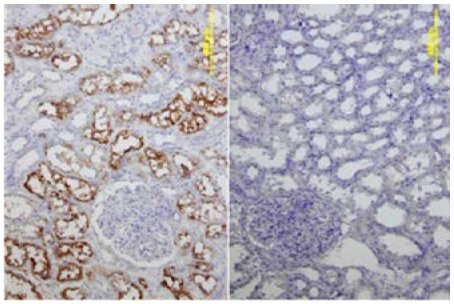


Figure 4. ACE-2 in Human Kidney. ACE-2 was detected in immersion-fixed paraffin-embedded sections of human kidney using a Goat Anti-Human ACE-2 Antigen Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF933). The tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (R&D Systems, Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents

RECOMBINANT PROTEINS

MOLECULE	BRAND	CATALOG #	SPECIES	SOURCE	TAG
ACE-2	R&D Systems	933-ZN	Human	NS0	No
	R&D Systems	3437-ZN	Mouse	CHO	No
Aminopeptidase N/CD13	R&D Systems	3815-ZN	Human	NS0	No
	R&D Systems	2335-ZN	Mouse	NS0	No
Cathepsin B (Native Protein)	Novus Biologicals	NBP1-99197	Human	Human liver	No
Cathepsin B	R&D Systems	953-CY	Human	NS0	No
	R&D Systems	965-CY	Mouse	NS0	No
	Novus Biologicals	NBP2-53084	Mouse	Baculovirus	No
Cathepsin L	R&D Systems	952-CY	Human	NS0	No
	R&D Systems	1515-CY	Mouse	NS0	No
CEACAM-1/CD66a	R&D Systems	2244-CM	Human	NS0	No
DDPIV/CD26 (High Purity Dimer)	R&D Systems	9168-SE	Human	NS0	No
DDPIV/CD26	R&D Systems	954-SE	Mouse	NS0	No
	R&D Systems	9637-SE	Cynomolgus Monkey	HEK293	No
EMMPRIN/CD147	R&D Systems	972-EMN	Human	NS0	No
	R&D Systems	772-EM	Mouse	NS0	His
Furin	R&D Systems	1503-SE	Human	NS0	No
	R&D Systems	6450-SE	Mouse	CHO	No
Ly6E	R&D Systems	9970-L6	Human	HEK293	No
Neutrophil Elastase/ELA2	R&D Systems	9167-SE	Human	CHO	No
	R&D Systems	4517-SE	Mouse	NS0	No
SARS-CoV-2 Papain-like Protease	R&D Systems	E-611	Virus	*E. coli*	No
TMPRSS2	Novus Biologicals	H00007113-Q01	Human	Wheat germ	No
TMPRSS2 Recombinant Protein Antigen	Novus Biologicals	NBP2-38263PEP	Human	*E. coli*	No
TMPRSS2 Overexpression Lysate	Novus Biologicals	NBL1-17121	Human	HEK293T	No

Learn more | rndsystems.com/proteins

ANTIBODIES

MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
ACE-2	Novus Biologicals	NBP2-67692*	Human, Mouse, Rat	SN0754	ICC/IF, IHC, IP, WB	No
	Novus Biologicals	NBP2-80035	Human	AC18F	ELISA, Flow, WB	No
	R&D Systems	MAB933	Human	171606	IHC, WB	No
	R&D Systems	MAB9331	Human	171608	IP, WB	No
	R&D Systems	AF933	Human	Poly	IHC, IP, SW, WB	Yes
	R&D Systems	AF3437	Mouse	Poly	IHC, IP, SW, WB	No
Aminopeptidase N/ CD13	Novus Biologicals	NBP2-77451	Human, Mouse, Rat	Poly	Flow, IHC, WB	Yes
	Novus Biologicals	NBP2-23492	Human, Mouse	R3-63	FA, Flow, ICC/IF, IHC	No
	R&D Systems	MAB3815	Human	498001	ICC/IF, IHC, SW, WB	No
	R&D Systems	AF3815	Human	Poly	CyTOF, Flow, IHC, IP, SW, WB	Yes
	Novus Biologicals	NB100-64843	Mouse	ER-BMDM1	Flow, IHC	Yes
	R&D Systems	AF2335	Mouse	Poly	CyTOF, Flow, ICC/IF, IHC, IP, WB	Yes
Cathepsin B	Novus Biologicals	NBP1-19797	Human, Mouse, Rat	Poly	ICC/IF, IHC, WB	Yes
	Novus Biologicals	NBP2-67215*	Human, Mouse	JA11-02	ICC/IF, IHC, WB	No
	R&D Systems	MAB965	Human, Mouse	173317	WB	No
	Novus Biologicals	NBP1-86048	Human	Poly	ICC/IF, IHC	No
	R&D Systems	AF953	Human	Poly	IHC, IP, KO, SW, WB	Yes
	Novus Biologicals	NBP1-25931	Human	Poly	ELISA	FITC
	R&D Systems	AF965	Mouse	Poly	B/N, IHC, WB	Yes
Cathepsin L	Novus Biologicals	NB100-1775	Human, Mouse, Rat +	33/2	ELISA, ICC/IF, IHC, MiAR, WB	No
	Novus Biologicals	NBP2-67216*	Human, Mouse, Rat	JM10-78	Flow, ICC/IF, IHC, WB	No
	R&D Systems	MAB9521	Human, Mouse	204101	IHC, IP, WB	No
	R&D Systems	MAB952	Human	204106	IHC, WB	No
	R&D Systems	AF952	Human	Poly	ELISA, IHC, IP, WB	Yes
	R&D Systems	AF1515	Mouse, Rat	Poly	IHC, SW, WB	Yes
CEACAM-1/CD66a	R&D Systems	MAB2244	Human	283340	CyTOF, Flow, WB	Yes
	R&D Systems	MAB22441	Human	283324	ELISA, IHC, WB	No
	R&D Systems	AF2244	Human	Poly	IHC, WB	Yes
	Novus Biologicals	NBP1-43390	Mouse	CC1	B/N, Flow, ICC/IF, IHC, IP, IVT, WB	Yes
	R&D Systems	AF6480	Mouse	Poly	CyTOF, Flow, IHC, WB	No
	Novus Biologicals	NBP2-59674	Rat	11-1H	ELISA, Flow, ICC/IF, IHC, IP, WB	Yes
DDPIV/CD26	Novus Biologicals	NB100-59021	Human +	Poly	IHC	No
	Novus Biologicals	NBP2-78791	Human, Mouse, Rat	JM11-42	ICC/IF, IHC, Flow, WB	No
	R&D Systems	MAB1180	Human	222113	CyTOF, ELISA, Flow, WB	Yes
	R&D Systems	AF1180	Human	Poly	ICC/IF, IHC, SW, WB	Yes
	R&D Systems	MAB9541	Mouse	155202	CyTOF, Flow	Yes
	R&D Systems	AF954	Mouse	Poly	IHC, SW, WB	Yes
	Novus Biologicals	NB100-61658	Rat	Poly	IHC, PEP-ELISA, WB	No
EMMPRIN/CD147	Novus Biologicals	NB500-430	Human +	MEM-M6/1	CyTOF, ELISA, Flow, IHC, IP, WB	Yes
	R&D Systems	MAB972	Human	109403	KO, WB	No
	R&D Systems	AF972	Human	Poly	IHC, KO, SW, WB	Yes
	R&D Systems	MAB7721*	Mouse	1159A	IHC, WB	Yes
	R&D Systems	MAB772	Mouse	116318	WB	No
	R&D Systems	AF772	Mouse	Poly	CyTOF, Flow, SW, WB	Yes
	Novus Biologicals	NB100-64848	Rat	OX-47	CyTOF, Flow, ICC/IF, IHC, WB	Yes
Furin	Novus Biologicals	NB100-1903	Human, Mouse, Rat +	Poly	B/N, Flow, ICC/IF, IHC, IP, WB	No
	Novus Biologicals	NBP2-75495*	Human, Mouse, Rat	JB35-53	ICC/IF, IHC, WB	No
	R&D Systems	MAB15032	Human	222712	ELISA, WB	No
	R&D Systems	AF1503	Human	Poly	IP, WB	Yes
	Novus Biologicals	NBP2-26104	Human	Poly	PEP-ELISA, WB	No

ANTIBODIES

MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
Ly6E	Novus Biologicals	NBP1-52176	Mouse	Poly	Flow, ICC/IF, PEP-ELISA	No
Neutrophil Elastase/ ELA2	Novus Biologicals	NBP2-61657	Human, Rat	6B6G6	ELISA, Flow, WB	No
	Novus Biologicals	NBP2-61658	Human +	6B6B10	ELISA, Flow, IHC, WB	No
	R&D Systems	MAB91671R*	Human	950317R	CyTOF, ICC/IF, IHC, Flow-IC, WB	Yes
	R&D Systems	MAB91671	Human	950317	CyTOF, Flow, ICC/IF, IHC, WB	Yes
	Novus Biologicals	NBP2-66972*	Human	JF098-6	Flow, ICC/IF, IHC, WB	No
	R&D Systems	MAB4517	Mouse	887105	WB	No
SARS Spike Protein	Novus Biologicals	NBP2-24942	Virus	17F706	WB	Yes
	Novus Biologicals	NBP2-24808	Virus	Poly	ELISA	No
	Novus Biologicals	NB100-56578	Virus	Poly	ICC/IF, WB	No
SARS-CoV 3CL Protease	Novus Biologicals	NBP1-78110	Virus	Poly	ELISA, ICC/IF, WB	No
SARS Nucleocapsid Protein	Novus Biologicals	NB100-56683	Virus	Poly	WB, ELISA, ICC/IF	No
	Novus Biologicals	NB100-56049	Virus	Poly	WB, ELISA	No
	Novus Biologicals	NBP2-24747	Virus	Ncap11	WB, ICC/IF	Yes
	Novus Biologicals	NBP2-24745	Virus	18F629.1	WB	No
SARS E2	Novus Biologicals	NBP1-28850	Virus	4A6C9	ELISA, WB	No
SARS Envelope Protein	Novus Biologicals	NB100-56562	Virus	Poly	WB, ELISA	No
SARS Membrane Protein	Novus Biologicals	NB100-56569	Virus	Poly	WB	No
	Novus Biologicals	NBP1-28852	Virus	2H2C4	ELISA, WB	No
SARS RDRP	Novus Biologicals	NBP2-50258	Virus	4E6	WB	Yes
TMPRSS2	Novus Biologicals	H00007113-M05	Human	2F4	WB, ELISA	No
	Novus Biologicals	NBP1-20984	Human	Poly	WB, PEP-ELISA	No
	Novus Biologicals	H00007113-B01P	Human	Poly	WB	No

PEPTIDE SUBSTRATES

PRODUCT	BRAND	CATALOG #	DESCRIPTION
Mca-YVADAPK(Dnp)-OH Fluorogenic Peptide Substrate	R&D Systems	ES007	Substrate for ACE-2 and Caspase-1
Z-LR-AMC Fluorogenic Peptide Substrate	R&D Systems	ES008	Substrate for Cathepsin B, Cathepsin L, and Cathepsin V

Species Key: + Additional Species Available

Applications Key: B/N Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **FA** Functional Assay, **Flow** Flow Cytometry, **Flow-IC** Flow Cytometry (Intracellular), **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **IVT** *In Vitro*, **KO** Knockout Validated, **MiAR** Microarray, **PEP-ELISA** Peptide ELISA, **SW** Simple Western™, **WB** Western blot

*Indicates a recombinant monoclonal antibody

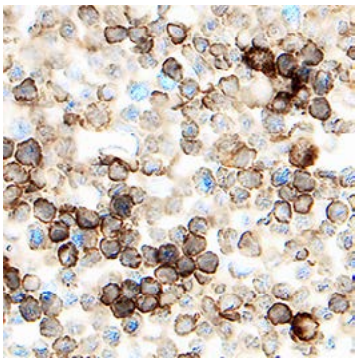


Figure 5. DPPIV/CD26 was detected in immersion fixed frozen sections of mouse thymus using Goat Anti-Mouse DPPIV/CD26 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF954). The tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. All cited reagents are from R&D Systems.

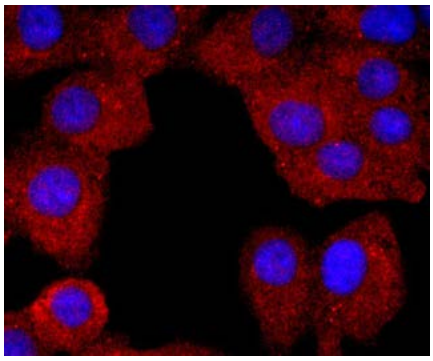


Figure 6. Neutrophil Elastase/ELA2 was detected in fixed A549 human lung carcinoma epithelial cells using a Rabbit Anti-Human Neutrophil Elastase/ELA2 Recombinant Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-66972). Cells were stained red and counterstained with DAPI (blue).

ELISAS				
MOLECULE	BRAND	SPECIES	CATALOG #	KIT TYPE
ACE-2	Novus Biologicals	Human	NBP2-66387	Chemiluminescence
	Novus Biologicals	Human	NBP2-78734	Colorimetric
Aminopeptidase N/CD13	R&D Systems	Human	DY3815	DuoSet®
Pro-Cathepsin B	R&D Systems	Human	DCATB0	Quantikine®
	R&D Systems	Human	DY953	DuoSet
Total Cathepsin B	R&D Systems	Human	DY2176	DuoSet
Cathepsin B	Novus Biologicals	Mouse	NBP2-67254	Colorimetric
	Novus Biologicals	Rat	NBP2-67255	Colorimetric
Cathepsin L	R&D Systems	Human	DY952	DuoSet
	Novus Biologicals	Mouse	NBP2-89172	Colorimetric
	Novus Biologicals	Rat	NBP2-89173	Colorimetric
CEACAM-1/CD66a	R&D Systems	Human	DY2244	DuoSet
EMMPRIN/CD147	R&D Systems	Human	DEMP00	Quantikine
	R&D Systems	Human	DY972	DuoSet
Furin	R&D Systems	Human	DY1503	DuoSet
	Novus Biologicals	Human	NBP2-67962	Colorimetric
Neutrophil Elastase/ELA2	R&D Systems	Mouse	DY4517	DuoSet
	R&D Systems	Human	DY9167	DuoSet
	R&D Systems	Mouse	MELA20	Quantikine
TMPRSS2	Novus Biologicals	Human	NBP2-89170	Colorimetric
	Novus Biologicals	Human	NBP2-89171	Chemiluminescence

SMALL MOLECULES			
PRODUCT	BRAND	CATALOG #	DESCRIPTION
CHR 2797	Tocris	3595	Aminopeptidase inhibitor, also called Tosedostat
MDL 28170	Tocris	1146	Potent and selective Calpain and Cathepsin B inhibitor
CA 074	Tocris	4863	Selective Cathepsin B inhibitor
Calpeptin	Tocris	0448	Calpain and Cathepsin L inhibitor
SID 26681509	Tocris	3625	Cathepsin L inhibitor
N-Acetyl-L-leucyl-L-leucyl-L-methional	Tocris	0384	Cathepsin inhibitor
E 64d	Tocris	4545	Cathepsin inhibitor; interferes with autolysosomal digestion
DPPI 1c	Tocris	2783	DPPIV/CD26 inhibitor
K 579	Tocris	2790	DPPIV/CD26 inhibitor
NVP DPP 728	Tocris	3506	Potent DPPIV/CD26 inhibitor; orally active
Saxagliptin	Tocris	6507	High affinity DPPIV/CD26 inhibitor; active *in vivo*
SSM 3	Tocris	5253	Potent Furin inhibitor
ONO 6818	Tocris	5651	High affinity and selective Human Neutrophil Elastase 1 (HNE1) inhibitor; orally active
BAY 678	Tocris	5706	Potent Human Neutrophil Elastase (HNE) inhibitor; cell permeable
BAY 677	Tocris	6389	Inactive control for BAY 678 (Catalog # 5706)
Camostat	Tocris	3193	TMPRSS2 inhibitor
Hydroxychloroquine sulfate	Tocris	5648	Autophagy inhibitor, also inhibits TLR9; inhibits SARS-CoV-2 viral infection

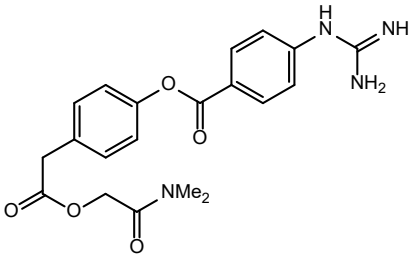
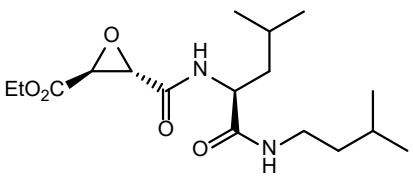


Figure 7. Camostat mesylate (Tocris, Catalog # 3193) is an orally active protease inhibitor. Hoffmann *et al.* showed that camostate mesylate can block SARS-CoV-2 infection of lung cells.

Figure 8. E 64d (Tocris, Catalog # 4545) is an inhibitor of Cathepsin B and L. Hoffmann *et al.* showed that E 64d can inhibit entry of SARS-CoV-2 into cells when combined with the TMPRSS2 inhibitor, Camostat mesylate.



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MONITORING DISEASE PROGRESSION AND TARGETING THE CYTOKINE STORM

According to reports from the General Office of National Health Commission Office of State TCM Administration: Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 6, Revised), severe and critically ill patients with SARS-CoV-2 often have elevated inflammatory factors. Similarly, a proinflammatory cytokine response has been associated with SARS-CoV infections. Therefore, analysis of inflammatory factors in severe patients may be used to monitor disease progression, disease trends, evaluate prognosis, and guide therapy.

Cytokines (including inflammatory cytokines) are signaling proteins involved in intercellular communication that are released by a broad range of cell types. Within the immune system, cytokines regulate immunological and inflammatory responses. Through the course of viral infections, overactivation of the immune system may lead to excessive production of immune cells and inflammatory cytokines, a process commonly referred to as a cytokine storm or cytokine release syndrome (CRS). SARS-CoV and MERS-CoV infections are frequently associated with inflammatory cellular pulmonary infiltration and elevated proinflammatory cytokines, which lead to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). In severe cases, local lung inflammation may lead to sepsis syndrome. The plasma cytokine profile of patients with pulmonary infection and subsequent sepsis show acute release of tumor necrosis factor- α (TNF-α), IL-β/IL-1F2, IL-8/CXCL8, and CCL2/monocyte chemoattractant protein-1 (MCP-1), followed by sustained release of IL-6. Release of IL-10 follows as the immune system attempts to control the inflammation.

For SARS-CoV-2 infection, recent epidemiological studies have proposed a correlation between elevated levels of inflammatory cytokines and disease severity. Patients infected with SARS-CoV-2 show high levels of IL-1β, IFN-γ, CXCL10/IP-10, and CCL2/MCP-1. Moreover, patients with severe symptoms show significantly higher levels of plasma proinflammatory factors (IL-2, IL-7, IL-10, G-CSF, CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1α, TNF-α) than patients with milder symptoms.

These findings suggest that, similar to patients infected with SARS-CoV and MERS-CoV, the levels of cytokine secretion may correlate with the severity of lung lesions in COVID-19 patients. Consequently, therapies that regulate the activity of inflammatory cytokines, such as neutralizing/blocking antibodies or inhibitors, may prove effective at alleviating tissue and organ damage caused by the cytokine storm. Known to suppress cytokine storms in cell therapy, Tocilizumab, a humanized monoclonal antibody against the IL-6 receptor (IL-6 R), has recently been registered in a multicenter, randomized controlled clinical study to evaluate its efficacy for treating COVID-19 (registration number: ChiCTR2000029765).

Cytokine storm monitoring plays a vital role in the evaluation of disease progression. Bio-Techne provides the most comprehensive resources for cytokine analysis and detection, including ELISA kits for individual cytokine detection, Luminex® kits for multiplex cytokine assays, antibody arrays, and automatic ELISA instruments for both quantitative and qualitative detection. Researchers can choose from a large selection of recombinant proteins and antibodies for blocking/neutralization. Biosimilar antibodies, which mimic the effects of existing monoclonal antibodies, and anti-idiotype antibodies, which can be used to evaluate the efficacy of existing antibody drugs, are available to study therapeutic monoclonal antibodies in inflammatory disease. Many of these antibodies are also validated for additional applications including flow cytometry, immunocytochemistry (ICC), IHC, and Western Blot.

PRODUCTS TO INVESTIGATE THERAPEUTIC MONOCLONAL ANTIBODIES

BIOSIMILAR ANTIBODIES

MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	THERAPEUTIC ANTIBODY	CONJUGATES AVAILABLE
IL-6 R	Novus Biologicals	NBP2-75192	Human	rhPM-1	Tocilizumab	No
IL-6 R	Novus Biologicals	NBP2-75193	Human	rhPM-1	Tocilizumab	No
IL-6 Rα	R&D Systems	MAB10346*	Human	Hu137	Sarilumab	No
TNF-α	R&D Systems	MAB9677	Human	Hu7	Adalimumab	Yes
Integrin α4β7/ LPAM-1	R&D Systems	MAB10078	Human	Hu117	Vedolizumab	Yes
CD25/IL-2 Rα	R&D Systems	MAB9926	Human	Hu107	Basiliximab	Yes
CD25/IL-2 Rα	R&D Systems	MAB9927	Human	Hu102	Daclizumab	Yes

ANTI-IDIOTYPE ANTIBODIES

ANTIBODY	BRAND	CATALOG #	CLONE	CONJUGATES AVAILABLE
Adalimumab	R&D Systems	MAB9616*	2235F	No
Adalimumab	R&D Systems	MAB9546	972557	No
Daclizumab	R&D Systems	MAB10218*	2498A	No

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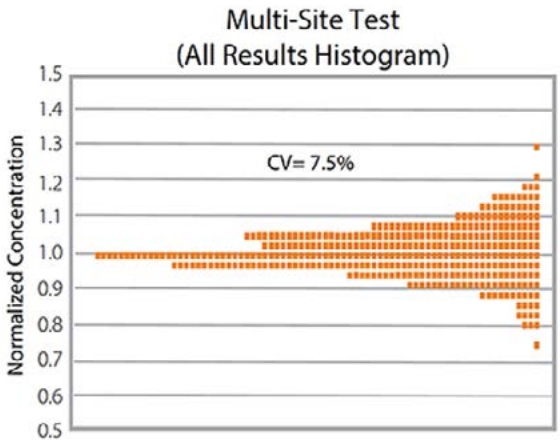
SIMPLE PLEX™ ASSAYS

Simple Plex assays are highly sensitive, reproducible and completely automated immunoassays that will transform your research possibilities. Run on the Ella™ platform, these novel assays split just 25 mL of sample volume across isolated microfluidic channels, allowing for the analysis of multiple analytes with no risk of cross-reactivity. Simple Plex assays exhibit the same specificity of a single plex ELISA, but with greater sensitivity and a broader dynamic range. The cartridges are also factory calibrated, which along with the reduction in manual steps, provides the highest quality data in just 90 minutes (Figure 9).



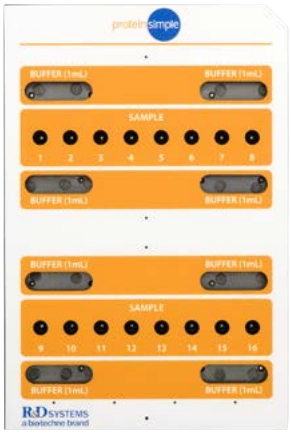


Figure 9. Testing across three sites, 11 users and nine Ella instruments. Four different assays were processed for CCL2, IL-6, TNF-α and VEGF-A. Eight unique serum samples with two controls for a total of 704 answers. The 16x4 cartridge used in this study is ideal for small batches of samples and provides highly reproducible multianalyte biomarker analysis in real-time.

BIOLOGICAL PHENOTYPING IN REAL-TIME

When researching an acute and deadly disease, speed and data quality are key. With the benefits of full automation, preloaded reagents, and factory calibration curves, Simple Plex assays on Ella provide speed, efficiency and high-quality sample data to support the rapid detection of a viral-induced immunologic response.

- Walk-away automated system, easy to use, minimal training
- Results in 70 minutes, with minimal sample preparation
- Waste contained in the cartridge
- Full LIMS integration
- Highly reproducible
- Rapid high-quality data for faster decisions

Customize your own multianalyte panel or measure single analytes by choosing from our extensive menu of assays.

ELLA COVID-19 PACKAGE

Ella is here to help your fight against COVID-19. A new 16x4 multianalyte cartridge is now available for the rapid analysis of key inflammatory markers. This panel of analytes has been put together in collaboration with, and per the request of Mt. Sinai Hospital (New York), and the Spallanzani Hospital & National Institute for Infectious Diseases in Italy (Rome).

COVID-19 CYTOKINE ANALYSIS PANEL	
PRODUCT CODE	ANALYTES DETECTED
SPCKA-PS-003229	IL-1β/IL-1F2, IL-6, IL-8/CXCL8, TNF-α

Along with this panel, we are offering researchers in this space the Ella platform and everything you need to get your experiments started straightaway. This package includes:

- The Ella Immunoassay Platform with Laptop
- 21 CFR Part 11 Software Toolset
- One Year Platinum Service
- Free Application Science Training
- 5x COVID-19 Cytokine Panel Cartridges

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LUMINEX ASSAYS

- Analyte flexibility with the largest, mix and match menu on the market
- Faster time to results with premixed beads sets
- Performance validated and tested
- Fast delivery times

LUMINEX HIGH PERFORMANCE ASSAYS

- Optimized panels
- Pick and choose analytes
- Pre-mixed for optimal performance
- Extensive menu of analytes
- Fast delivery times

LUMINEX HIGH PERFORMANCE ASSAYS		
PRODUCT	MAXIMUM # ANALYTES DETECTED	CATALOG #
Human XL Cytokine High Performance Panel	45	FCSTM18
CCL2/JE/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/Eotaxin, CCL19/MIP-3β, CCL20/MIP-3α, CD40 Ligand/TNFSF5, CX3CL1/Fractalkine, CXCL1/GROα/KC/CINC-1, CXCL2/GROβ/MIP-2/CINC-3, CXCL10/IP-10, FGF basic/FGF2/bFGF, Flt-3 Ligand/FLT3L, G-CSF, GM-CSF, Granzyme B, IFN-α/IFNA2, IFN-β, IFN-γ, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7		
Human High Sensitivity Cytokine Panel A	12	FCSTM09
GM-CSF, IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-8/CXCL8, IL-10, IL-12 p70, TNF-α, VEGF		
Human High Sensitivity Cytokine Panel B	17	FCSTM14
GM-CSF, IFN-γ, IL-1β/IL-1F2, IL-2, IL-5, IL-6, IL-7, IL-13, IL-15, IL-17A, IL-17F, IL-22, IL-23, IL-31, IL-33, IL-36β, TNF-α		
Human Fixed Th1/Th2 Discovery 11-plex	11	LKTM008
GM-CSF, IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, IL-13, TNF-α		
NHP XL Cytokine High Performance Panel	35	FCSTM21
BDNF, CCL2/MCP-1, CCL5/RANTES, CCL11/Eotaxin, CCL20/MIP-3α, CD40 Ligand/TNFSF5, CXCL2/GROβ, CXCL10/IP-10, CXCL11/I-TAC, CXCL13/BLC, FGF basic/FGF2/bFGF, G-CSF, GM-CSF, Granzyme B, IFN-α, IFN-β, IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, IL-21, PDGF-AA, PDGF-BB, PD-L1, TGF-α, TNF-α		

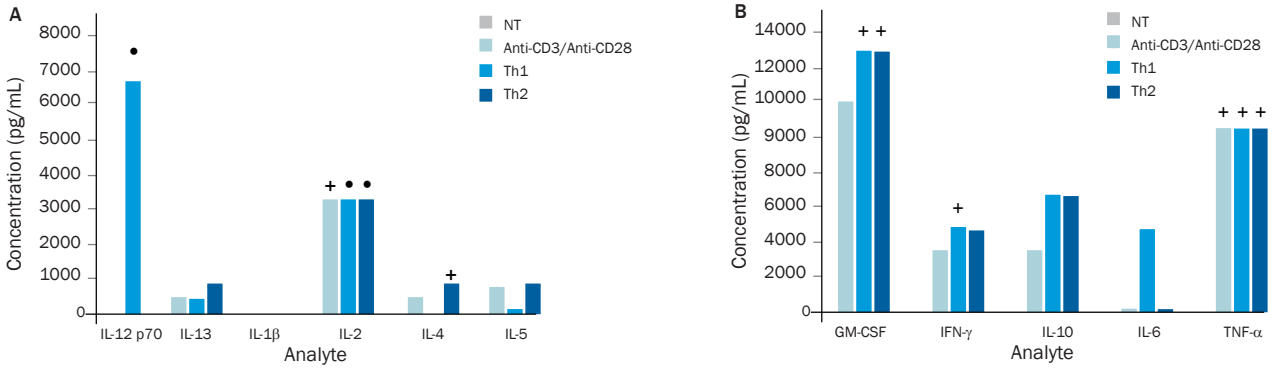

Figure 10. Quantitation of Cytokines in Cell Culture Supernates from Activated Th1 and Th2 Cells. $CD4^+$ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using the MagCellect Human $CD4^+$ T Cell Isolation Kit (R&D Systems, Catalog # MAGH102). Cells were either untreated (NT), treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL), Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL), and Mouse Anti-Human IL-4 Monoclonal Antibody (R&D Systems, Catalog # MAB304) and activated with immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (R&D Systems, Catalog # MAB100) and soluble Mouse Anti-Human CD28 Monoclonal Antibody (R&D Systems, Catalog # MAB342) to induce Th1 differentiation, or treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL) and activated with Phytohemagglutinin-L (PHA) to induce Th2 differentiation. All stimulated cells were then treated with PMA (Tocris, Catalog # 1201; 10 ng/mL) and Ionomycin calcium salt (Tocris, Catalog # 1704; 500 ng/mL) for 24 hours after activation. Cell culture supernates were analyzed using the Magnetic Luminex Performance Human Fixed Th1/Th2 Discovery 11-plex Panel (R&D Systems, Catalog # LKTM008).

+ = Values above the limits of the standard curve • = Stimulating cytokine

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PROTEOME PROFILER™ ANTIBODY ARRAYS

R&D Systems™ Proteome Profiler Antibody Arrays offer a quick and inexpensive analysis of many analytes simultaneously, in less time than it takes to perform a Western blot (Figure 11-12). Highly cited and rated 5 stars by our customers, these membrane-based arrays are ideal for detecting cytokines released following viral infection.

- Cost-effective analyte profiling
- No specialized equipment needed, use standard Western blot data collection equipment
- Superior performance
- Multiple analyte results in only 5 ½ hours
- Multiple detection methods available*

*Select arrays only

PROTEOME PROFILER ANTIBODY ARRAYS			
PRODUCTS	DESCRIPTION	CATALOG #	SIZE
Human XL Cytokine Array Kit	Contains 4 membranes - each spotted in duplicate with 105 different cytokine antibodies	ARY022B	1 Kit
Adiponectin/Acrp30, Angiogenin, Angiopoietin-1, Angiopoietin-2, Apolipoprotein A1, BAFF/BLyS/TNFSF13B, BDNF, CCL2/JE/MCP-1, CCL3/CCL4 (MIP-1α/MIP-1β), CCL5/RANTES, CCL-7/MCP-3, CCL17/TARC, CCL19/MIP-3β, CCL20/MIP-3α, CD14, CD30 CD40/TNFRSF5, Chitinase 3-like, Complement Component C5/C5a, Complement Factor D, C-Reactive Protein/CRP, Cripto-1, CXCL1/GROα, CXCL4/PF4, CXCL5/ENA-78, CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CXCL12/SDF-1α, Cystatin C, Dkk-1, DPPIV/CD26, EGF, EMMPRIN, Endoglin/CD105, Fas Ligand, FGF basic/FGF2/bFGF, FGF-19, Flt-3 Ligand, G-CSF, GDF-15, GM-CSF, Growth Hormone, HGF, ICAM-1/CD54, IFN-γ, IGFBP-2, IGFBP-3, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-12 p70, IL-13, IL-15, IL-16, IL-17A, IL-18 BPz, IL-19, IL-22, IL-23, IL-24, IL-27, IL-28, IL-31, IL-32 α/β/γ, IL-33, IL-34, Kallikrein 3/PSA, KGF/FGF-7, Leptin, LIF, Lipocalin-2/NGAL, M-CSF, MIF, MMP-9, Myeloperoxidase, Osteopontin (OPN), PDGF-AA, PDGF-AB/BB, Pentraxin 3/TSF-14, RAGE, RBP4, Relaxin-2, Resistin, Serpin E1/PAI-1, SHBG, ST2/IL-1 R4, TFF3, TfR, TGF-α, Thrombopoietin, TIM-1/KIM-1/HAVCR, TNF-α, uPAR, VCAM-1/CD106, VEGF, Vitamin D BP			
Human Cytokine Array Kit	Contains 4 arrays - each spotted in duplicate with 36 different cytokine antibodies	ARY005B	1 Kit
CCL1/I-309, CCL2/MCP-1, CCL3/CCL4 (MIP-1α/MIP-1β), CCL5/RANTES, CD40 Ligand/TNFSF5, CXCL1/GROα, CXCL8/IL-8, CXCL10/IP-10, CXCL11/I-TAC, CXCL12/SDF-1α, G-CSF, GM-CSF, ICAM-1/CD54, IFN-γ, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-13, IL-16, IL-17, IL-17E/IL-25, IL-18, IL-21, IL-27, IL-32α, MIF, Serpin E1/PAI-1, TNF-α, TREM-1			

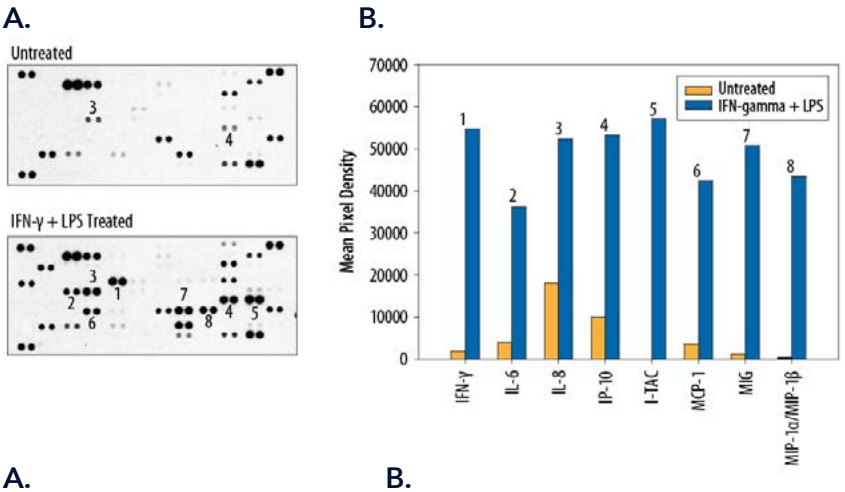

Figure 11. THP-1 human acute monocytic leukemia cells were treated with Recombinant Human IFN-γ (Catalog # 285-IF) for 16 hours, followed by LPS for 8 hours, or remained untreated. Lysates from untreated and treated cells were examined for the levels of 105 different cytokines using the Proteome Profiler Human XL Cytokine Antibody Array (Catalog # ARY022B). Representative arrays (**A**) and histogram profiles (**B**) for select analytes from untreated (orange) and treated (blue) cells. All cited reagents are from R&D Systems.

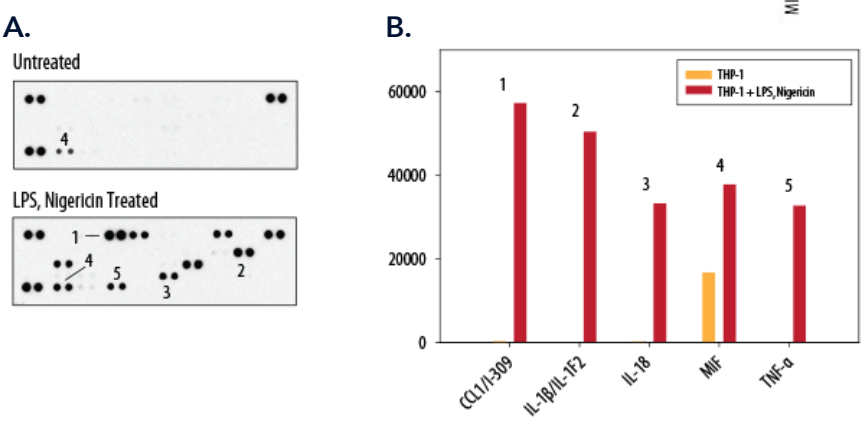

Figure 12. THP-1 human acute monocytic leukemia cells were treated with LPS for 4 hours, followed by the selective K+ ionophore Nigericin (Tocris, Catalog # 4312), or remained untreated. Lysates from untreated and treated cells were examined for the levels of 36 different cytokines using the Proteome Profiler Human Cytokine Antibody Array (R&D Systems, Catalog # ARY005B). Representative arrays (**A**) and histogram profiles (**B**) for select analytes from untreated (orange) and treated (red) cells.

Learn more | rndsystems.com/proteomeprofiler

ELISAS

Bio-Techne has over 30 years of experience in designing, testing, and optimizing immunoassay kits. Our R&D Systems™ brand ELISAs are the most trusted and most published ELISAs on the market (Figures 13-14). We currently offer kits for 700 target analytes spanning 12 species. R&D Systems Quantikine® ELISA Kits are complete, fully validated, ready-to-run sandwich ELISAs. They are manufactured in-house and undergo extensive validation testing to ensure that they perform as expected every time. R&D Systems™ DuoSet® ELISA Development Systems allow you to develop the immunoassay that you need using our gold-standard ELISA reagents. They are an economical alternative to buying separate antibodies and protein standards when complete kits are not an option.

DUOSET ELISAS

- Contains carefully selected and validated antibodies, reducing development time
- Includes mass-calibrated recombinant standard, reducing assay variability
- Adaptable to work with multiple platforms, such as Gyrolab™, MSD®, and Luminex®
- The largest selection of analytes (>1000) for the most species
- Economical alternative as many analytes are offered in a smaller 5-plate pack

QUANTIKINE ELISAS

- Most referenced on the market
- Complete, ready-to-use kits
- Undergoes rigorous validation testing to ensure superior quality and reproducibility
- Accurate detection of natural proteins
- Colorimetric detection

QUANTIKINE AND DUOSET ELISAS

ANALYTE	BRAND	SPECIES	QUANTIKINE CATALOG #	DUOSET CATALOG #
CCL2/MCP-1	R&D Systems	Human	DCP00	DY279
CCL2/JE/MCP-1	R&D Systems	Mouse	MJE00B	DY479
	R&D Systems	Rat		DY3144
CCL3/MIP-1α	R&D Systems	Human	DMA00	DY270
	R&D Systems	Mouse	MMA00	DY450
CCL4/MIP-1β	R&D Systems	Human	DMB00	DY271
	R&D Systems	Mouse	MMB00	DY451
CCL5/RANTES	R&D Systems	Human	DRN00B	DY278
	R&D Systems	Mouse, Rat	MMR00	DY478
CHI3L1/YKL-40	R&D Systems	Human	DC3L10	DY2599
	R&D Systems	Mouse	MC3L10	DY2649
CX3CL1/Fractalkine	R&D Systems	Human	DCX310	DY365
	R&D Systems	Mouse	MCX310	DY472
	R&D Systems	Rat		DY537
CXCL1/GROα	R&D Systems	Human	DGR00B	DY275
CXCL1/KC	R&D Systems	Mouse	MKC00B	DY453
CXCL1/CINC-1	R&D Systems	Rat	RCN100	DY515
CXCL9/MIG	R&D Systems	Human	DCX900	DY392
	R&D Systems	Mouse	MCX900	DY492
CXCL10/IP-10	R&D Systems	Human	DIP100	DY266
CXCL10/IP-10/CRG-2	R&D Systems	Mouse		DY466
GM-CSF	R&D Systems	Human*	DGM00	DY215
	R&D Systems	Mouse	MGM00	DY415
	R&D Systems	Rat		DY518
IFN-α 2/IFNA2	R&D Systems	Human		DY9345
IFN-β	R&D Systems	Human	DIFNB0	DY814
	R&D Systems	Mouse	MIFNB0	DY8234
IFN-γ	R&D Systems	Human	DIF50C	DY285B
	R&D Systems	Mouse	MIF00	DY485
	R&D Systems	Rat	RIF00	DY585
IL-1β/IL-1F2	R&D Systems	Human*	DLB50	DY201
	R&D Systems	Mouse	MLB00C	DY401
	R&D Systems	Rat	RLB00	DY501

QUANTIKINE AND DUOSET ELISAS				
ANALYTE	BRAND	SPECIES	QUANTIKINE CATALOG #	DUOSET CATALOG #
IL-1ra/IL-1F3	R&D Systems	Human	DRA00B	DY280
	R&D Systems	Mouse	MRA00	DY480
IL-2	R&D Systems	Human*	D2050	DY202
	R&D Systems	Mouse	M2000	DY402
	R&D Systems	Rat	R2000	DY502
IL-4	R&D Systems	Human*	D4050	DY204
	R&D Systems	Mouse	M4000B	DY404
	R&D Systems	Rat	R4000	DY504
IL-6	R&D Systems	Human*	D6050	DY206
	R&D Systems	Mouse	M6000B	DY406
	R&D Systems	Rat	R6000B	DY506
IL-8/CXCL8	R&D Systems	Human*	D8000C	DY208
IL-10	R&D Systems	Human*	D1000B	DY217B
	R&D Systems	Mouse	M1000B	DY417
	R&D Systems	Rat	R1000	DY522
IL-12 p70	R&D Systems	Human	D1200	DY1270
	R&D Systems	Mouse	M1270	DY419
IL-15	R&D Systems	Human	D1500	DY247
	R&D Systems	Mouse		DY447
IL-17/IL-17A	R&D Systems	Human*	D1700	DY317
	R&D Systems	Mouse	M1700	DY421
IL-17A/F Heterodimer	R&D Systems	Human		DY5194
	R&D Systems	Mouse	M17AF0	DY5390
IL-18/IL-1F4	R&D Systems	Human	DL180	DY318
IL-23	R&D Systems	Human	D2300B	DY1290
	R&D Systems	Mouse	M2300	DY1887
TGF-β1	R&D Systems	Human	DB100B	DY240
	R&D Systems	Mouse Rat +	MB100B	DY1679
TNF-α	R&D Systems	Human*	DTA00D	DY210
	R&D Systems	Mouse*	MTA00B	DY410
	R&D Systems	Rat	RTA00	DY510

*High sensitivity Quantikine ELISAs are available for these analytes.

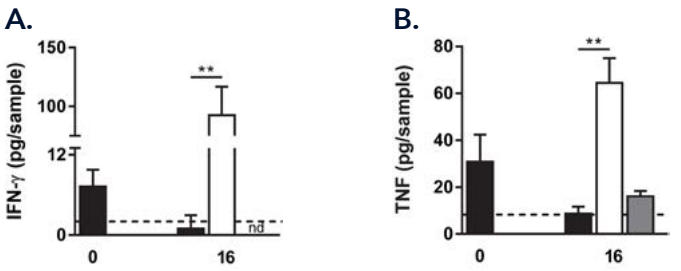

Figure 13. BALB/c mice were infected subcutaneously in the left footpad with *Mycobacterium ulcerans* stain 98-912. After development of a macroscopic lesion, mice were given two doses (s.c. injection) of Lysin B at 10 and 13 days post-infection. Levels of IFN-γ (**A**) and TNF-α (**B**) were quantified in the draining lymph node from non-treated *M. ulcerans* infected mice (black bars), treated *M. ulcerans* infected mice (white bars), and non-infected mice (grey bars) using the R&D Systems Mouse IFN-γ (Catalog # MIF00) and TNF-α (Catalog # MTA00B) Quantikine ELISA Kits. *** p≤0.01. Graph adapted from Fraga, A.G. et al. (2019) PLoS Negl. Trop. Dis.* ***13***:*e0007113.*

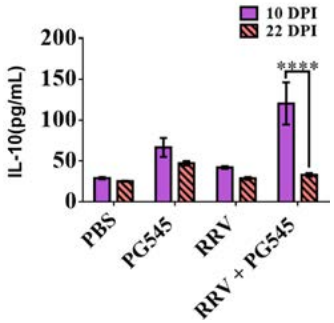

Figure 14. C57BL/6 mice were infected with the Ross River virus (RRV-T48 strain) or injected with PBS as a control. Animals were treated with PG545 (pixatimod), a cholestanol-conjugated, small molecule, heparan sulfate mimetic, or PBS (control) 1 day pre-infection and 4 and 9 days post-infection. Serum levels of IL-10 were analyzed 10 (solid bars) and 22 (stripped bars) days post-infection using the Mouse IL-10 DuoSet ELISA Development System (R&D Systems, Catalog # DY401). *****p<0.0001. Graph adapted from Supramaniam, A. et al. (2019) PLoS ONE* ***14***:*e0217998.*

Learn more | rndsystems.com/elisas

ADDITIONAL CYTOKINE-RELATED PRODUCTS

RECOMBINANT PROTEINS					
MOLECULE	BRAND	CATALOG #	SPECIES	SOURCE	TAG
CCL2/MCP-1	R&D Systems	279-MC	Human	*E. coli*	No
	Novus Biologicals	NBP1-81837PEP	Human	*E. coli*	No
CCL2/JE/MCP-1	R&D Systems	479-JE	Mouse	*E. coli*	No
CCL3/MIP-1α	R&D Systems	270-LD	Human	*E. coli*	No
	R&D Systems	450-MA	Mouse	*E. coli*	No
CXCL10/IP-10	R&D Systems	266-IP	Human	*E. coli*	No
CXCL10/IP-10/CRG-2	R&D Systems	466-CR	Mouse	*E. coli*	No
G-CSF	R&D Systems	214-CS	Human	*E. coli*	No
	R&D Systems	414-CS	Mouse	*E. coli*	No
IFN-γ	R&D Systems	285-IF	Human	*E. coli*	No
	R&D Systems	285-GMP	Human	*E. coli*	No
	R&D Systems	485-MI	Mouse	*E. coli*	No
IL-1β/IL-1F2	R&D Systems	201-LB	Human	*E. coli*	No
	R&D Systems	201-GMP	Human	*E. coli*	No
	R&D Systems	401-ML	Mouse	HEK293T	No
IL-4	R&D Systems	204-IL	Human	*E. coli*	No
	R&D Systems	204-GMP	Human	*E. coli*	No
	R&D Systems	404-ML	Mouse	*E. coli*	No
IL-7	R&D Systems	207-IL	Human	*E. coli*	No
	R&D Systems	207-GMP	Human	*E. coli*	No
	R&D Systems	407-ML	Mouse	*E. coli*	No
TNF-α	R&D Systems	210-TA	Human	*E. coli*	No
	R&D Systems	210-GMP	Human	*E. coli*	No
	R&D Systems	410-MT	Mouse	*E. coli*	No
VEGF	R&D Systems	293-VE	Human	*Sf* 21	No
	R&D Systems	493-MV	Mouse	*Sf* 21	No

ANTIBODIES						
MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
CCL2/MCP-1	Novus Biologicals	NBP2-22115	Human, Mouse, Rat +	2D8	ELISA, Flow, ICC/IF, IHC, WB	Yes
	Novus Biologicals	NBP1-07035	Human, Mouse, Rat	Poly	B/N, ICC/IF, IHC, WB	Yes
	R&D Systems	MAB679	Human	23007	B/N, ELISA, IHC, WB	No
	R&D Systems	AF-279-NA	Human	Poly	B/N, IHC, WB	Yes
	R&D Systems	AB-479-NA	Mouse	Poly	B/N, ELISA, WB	Yes
CCL3/MIP-1α	R&D Systems	MAB670	Human	14215	B/N, ELISA	No
	R&D Systems	AF-270-NA	Human	Poly	B/N, ELISA, IHC, WB	Yes
	R&D Systems	AF-450-NA	Mouse	Poly	B/N, ELISA, ICC/IF, IHC, WB	Yes
CXCL10/IP-10	R&D Systems	MAB266	Human	33036	B/N, CyTOF, ELISA, Flow	Yes
	R&D Systems	AF-266-NA	Human	Poly	B/N, CyTOF, ELISA, Flow, ICC/IF, IHC	Yes
CXCL10/IP-10/CRG-2	R&D Systems	AF-466-NA	Mouse	Poly	B/N, IHC, WB	Yes

ANTIBODIES

MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
G-CSF	R&D Systems	MAB214	Human	3316	B/N, ELISA	No
	R&D Systems	AF-214-NA	Human	Poly	B/N, WB	Yes
	R&D Systems	MAB414	Mouse	67604	B/N, ELISA, Flow, WB	Yes
IFN-γ	R&D Systems	MAB285	Human	25718	B/N, Flow, ICC/IF	Yes
	R&D Systems	MAB2852	Human	K3.53	B/N, ELISA, Flow, WB	Yes
	R&D Systems	MAB485	Mouse	37895	B/N, CyTOF, Flow, WB	Yes
IL-1 β/IL-1F2	Novus Biologicals	NBP1-19775	Human, Mouse, Rat	Poly	ICC/IF, IHC, WB	Yes
	Novus Biologicals	NBP2-27345	Human	43N3D8	Flow, IHC, WB	Yes
	R&D Systems	MAB601	Human	2805	B/N, ELISA, ICC/IF, WB	No
	R&D Systems	AF-201-NA	Human	Poly	B/N, ICC/IF, IHC, ISH-IHC,WB	Yes
	R&D Systems	AF-401-NA	Mouse	Poly	B/N, ICC/IF, IHC, SW, WB	Yes
IL-4	R&D Systems	MAB204	Human	34019	B/N, WB	No
	R&D Systems	MAB304	Human	3007	B/N, ICC/IF, WB	Yes
	R&D Systems	MAB404	Mouse	30340	B/N, ELISA, WB	No
IL-7	R&D Systems	MAB207	Human	7417	B/N, ELISA, WB	No
	R&D Systems	AF-207-NA	Human	Poly	B/N, WB	Yes
	R&D Systems	AF407	Mouse	Poly	B/N, ELISA, WB	Yes
TNF-α	Novus Biologicals	NBP1-19532	Human, Mouse, Rat +	Poly	Flow, ICC/IF, IHC, WB	Yes
	R&D Systems	AF-410-NA	Human, Mouse	Poly	B/N, CyTOF, ELISA, Flow, ICC/IF, WB	Yes
	R&D Systems	MAB610	Human	28401	B/N, ELISA, ICC/IF, WB	Yes
	R&D Systems	AB-410-NA	Mouse	Poly	B/N, WB	No
VEGF	R&D Systems	MAB293	Human +	26503	B/N, ELISA, WB	No
	R&D Systems	AF-293-NA	Human	Poly	B/N, ICC/IF, IHC, WB	Yes
	R&D Systems	AF-493-NA	Mouse	Poly	B/N, ELISA, IHC, WB	Yes

SMALL MOLECULES

PRODUCT	BRAND	CATALOG #	DESCRIPTION
AF 12198	Tocris	1793	Potent, selective human type I IL-1 receptor antagonist
CP 424174	Tocris	6107	Inhibitor of IL-1β post-translational processing; indirectly inhibits NLRP3
(D)-(+)-Neopterin	Tocris	4656	Stimulated by IFN-γ; marker of immune activation
(±)-AMG 487	Tocris	4487	CXCR3 antagonist; inhibits cell migration and metastasis
(±)-NBI 74330	Tocris	4528	Potent and selective CXCR3 antagonist
VUF 11222	Tocris	5668	High affinity non-peptide CXCR3 agonist
C 87	Tocris	5484	TNF-α inhibitor
R 7050	Tocris	5432	Inhibitor of TNF-α receptor 1 signaling
SKF 86002	Tocris	2008	Inhibits human monocyte IL-1 and TNF-α production; p38 MAP kinase inhibitor
GIT 27	Tocris	3270	Immunomodulator; reduces production of pro-inflammatory cytokines
JTE 607	Tocris	5185	Cytokine release inhibitor; anti-inflammatory
Axitinib	Tocris	4350	Potent VEGFR-1, -2 and -3 inhibitor
Ki 8751	Tocris	2542	Potent, selective VEGFR-2 inhibitor

Species Key: + Additional Species Available

Applications Key: B/N Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **Flow** Flow Cytometry, **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **ISH-IHC** Dual ISH-IHC, **SW** Simple Western™, **WB** Western blot

*Indicates a recombinant monoclonal antibody

PRODUCTS FOR ISOLATING AND IDENTIFYING IMMUNE CELLS

MAGCELLECT™ CELL SELECTION KITS & REAGENTS

In order to obtain a pure population of immune cells, researchers must often isolate or enrich the cell type of interest from a heterogeneous cell population. R&D Systems™ MagCellect Cell Selection Kits are designed to enrich for specific cell populations based on either a negative or positive selection principle.

MAGCELLECT CELL SELECTION KITS & REAGENTS

PRODUCT	SPECIES	CATALOG #	BRAND
CD14$^+$ Cell Isolation Kit	Human	MAGH105	R&D Systems
CD45$^+$ Cell Isolation Kit	Human	MAGH125	R&D Systems
CD3$^+$ T Cell Isolation Kit	Human	MAGH101	R&D Systems
	Mouse	MAGM201	R&D Systems
	Rat	MAGR301B	R&D Systems
Naïve CD4$^+$ T Cell Isolation Kit	Human	MAGH115	R&D Systems
	Mouse	MAGM205	R&D Systems
CD4$^+$ T Cell Isolation Kit	Human	MAGH102	R&D Systems
	Mouse	MAGM202	R&D Systems
	Rat	MAGR302B	R&D Systems
Memory CD4$^+$ T Cell Isolation Kit	Human	MAGH116	R&D Systems
	Mouse	MAGM206	R&D Systems
Naïve CD8$^+$ T Cell Isolation Kit	Mouse	MAGM207	R&D Systems
CD8$^+$ T Cell Isolation Kit	Human	MAGH112	R&D Systems
	Mouse	MAGM203	R&D Systems
Natural Killer Cell Isolation Kit	Human	MAGH109	R&D Systems
	Mouse	MAGM210	R&D Systems
MagCellect Magnet	N/A	MAG997	R&D Systems
MagCellect Streptavidin Ferrofluid	N/A	MAG999B	R&D Systems

CELLXVIVO™ KITS FOR IMMUNE CELL DIFFERENTIATION OR EXPANSION

Bio-Techne's CellXVivo Immune Cell Differentiation or Expansion Kits contain optimized combinations of the highest quality proteins and antibodies, along with simple, reproducible protocols for the differentiation or expansion of a variety of immune cell types including dendritic cells, macrophages, T helper cell subtypes, and natural killer cells.

CELLXVIVO KITS

PRODUCT	SPECIES	CATALOG #	BRAND
Dendritic Cell Differentiation Kit	Mouse	CDK008	R&D Systems
M1 Macrophage Differentiation Kit	Human	CDK012	R&D Systems
M2 Macrophage Differentiation Kit	Human	CDK013	R&D Systems
Monocyte-derived DC Differentiation Kit	Human	CDK004	R&D Systems
Natural Killer Cell Expansion Kit	Human	CDK015	R&D Systems
Th1 Cell Differentiation Kit	Human	CDK001	R&D Systems
	Mouse	CDK018	R&D Systems
Th17 Cell Differentiation Kit	Mouse	CDK017	R&D Systems

CLOUDZ™ CELL EXPANSION KITS

Bio-Techne's Cloudz Cell Expansion Kits utilize our pioneering Cloudz dissolvable hydrogel for the activation and expansion of specific immune cell populations. Using these dissolvable microspheres eliminates the need to use magnetic particles.

CLOUDZ KITS			
PRODUCT	SPECIES	CATALOG #	BRAND
Natural Killer Cell Expansion Kit	Human	CLD004	R&D Systems
Treg Expansion Kit	Human	CLD006	R&D Systems

ANTIBODIES FOR IMMUNE CELL IDENTIFICATION

Flow cytometry is widely used to identify and characterize different immune cell types in heterogeneous samples. It primarily relies on the use of fluorochrome-conjugated antibodies to detect the expression of specific cell surface or intracellular antigens on single cells in suspension. Bio-Techne offers an unparalleled selection of fluorochrome-conjugated R&D Systems and Novus Biologicals antibodies qualified for flow cytometry (Figures 15-17). Hundreds of world-renowned unique clones are available from the R&D Systems brand, many of which have been used to establish CD nomenclature through HLDA workshops. Additionally, the Novus Biologicals brand includes an expansive collection of both proprietary antibodies and some of the most highly referenced antibody clones on the market.

FLOW CYTOMETRY-VALIDATED ANTIBODIES				
CELL SURFACE MOLECULES	SPECIES	CLONE	BRAND	FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME)
NAÏVE T CELLS				
CD3$^+$	Human	UCHT1	R&D Systems	FAB100-A, C, F, G, N, P, R, S, T, U, V
	Mouse	17A2	R&D Systems	FAB4841-A, C, F, G, N, P, R, S, T, U, V
	Mouse	145-2C11	Novus Biologicals	NBP2-52641*
CD45RA$^+$	Human	MEM-56	Novus Biologicals	NB500-329-A, C, F, G, N, P, R, S, T, U, V
CD45RO$^-$	Human	UCHL-1	Novus Biologicals	NBP2-33104
CD62L/L-Selectin$^+$	Human +	FMC46	Novus Biologicals	NB100-65388
	Human	DREG56	Novus Biologicals	NBP1-42795
	Human	4G8	R&D Systems	FAB9787-G, R, T
	Mouse	MEL-14	Novus Biologicals	NBP2-81083*
CCR7$^+$	Human	3D12	Novus Biologicals	NBP1-43332
	Human	150503	R&D Systems	FAB197-A, F, G, N, P, R, S, T, U, V
	Human	150503R	R&D Systems	FAB197R-G, N, R, S, T, U, V*
	Mouse	4B12	R&D Systems	FAB3477-A, G, N, P, R, S, T, U, V
TH1 CELLS				
CD3$^+$	Human	UCHT1	R&D Systems	FAB100-A, C, F, G, N, P, R, S, T, U, V
	Mouse	17A2	R&D Systems	FAB4841-A, C, F, G, N, P, R, S, T, U, V
	Mouse	145-2C11	Novus Biologicals	NBP2-52641*
CD4$^+$	Human	RPA-T4	Novus Biologicals	NBP2-25199
	Human	13B8.2	Novus Biologicals	NBP2-52670*
	Human	11830	R&D Systems	FAB3791-A, C, F, G, N, P, R, S, T, V
	Mouse	GK1.5	R&D Systems	FAB554-A, C, F, G, N, P, R, S, T, U, V
T-bet/TBX21$^+$	Human, Mouse +	4B10	Novus Biologicals	NBP1-43298
	Human	525831	R&D Systems	FAB53851-G, N, R, S, T, U, V *
IFN-γ^+	Human	25723	R&D Systems	IC285-A, C, F, G, N, P, R, S, T, U, V
TNF-α^+ (membrane form)	Human, Mouse +	52B83	Novus Biologicals	NB600-1422
	Human	6401	R&D Systems	FAB210-F, P
	Human	6402	R&D Systems	IC210-F, P

FLOW CYTOMETRY-VALIDATED ANTIBODIES				
CELL SURFACE MOLECULES	SPECIES	CLONE	BRAND	FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME)
TH17 CELLS				
CD3+	Human	UCHT1	R&D Systems	FAB100-A, C, F, G, N, P, R, S, T, U, V
	Mouse	17A2	R&D Systems	FAB4841-A, C, F, G, N, P, R, S, T, U, V
	Mouse	145-2C11	Novus Biologicals	NBP2-52641*
CD4+	Human	RPA-T4	Novus Biologicals	NBP2-25199
	Human	13B8.2	Novus Biologicals	NBP2-52670*
	Human	11830	R&D Systems	FAB3791-A, C, F, G, N, P, R, S, T, V
	Mouse	GK1.5	R&D Systems	FAB554-A, C, F, G, N, P, R, S, T, U, V
CCR4+	Human	205410	R&D Systems	FAB1567-A, C, F, G, N, P, R, S, T, U, V
CCR6+	Human	53103	R&D Systems	FAB195-A, C, F, G, N, P, R, S, T, U, V
	Human	53103R	R&D Systems	FAB195R-G, N, R, S, T, U, V
	Mouse	140706	R&D Systems	FAB590-A, G, N, P, R, S, T, U, V
	Mouse	140706R	R&D Systems	FAB590R-G, N, R, S, T, U, V*
CCR10/GPR2-	Human	314305	R&D Systems	FAB3478-A, G, N, P, R, S, T, U, V
	Human	314305R	R&D Systems	FAB3478R-G, N, R, S, T, U, V*
	Mouse	248918	R&D Systems	FAB2815-A, C, G, N, P, R, S, T, U, V
	Mouse	248918R	R&D Systems	FAB2815R-G, N, R, S, T, U, V*
RORα/NR1F1	Human	784651	R&D Systems	IC8924-G, N, P, R, S, T, U, V
RORγt/RORC2/NRIF3+	Human, Mouse	600380	R&D Systems	IC6006-A, C, P
	Human	1181A	R&D Systems	IC9125-A, G, N, R, S, T, U, V *
IL-17/17A+	Human	41802	R&D Systems	IC3171-A, C, G, N, P, R, S, T, U, V
	Human	41809	R&D Systems	IC317-A, G, N, P
CYTOTOXIC T CELLS				
CD3+	Human	UCHT1	R&D Systems	FAB100-A, C, F, G, N, P, R, S, T, U, V
	Mouse	17A2	R&D Systems	FAB4841-A, C, F, G, N, P, R, S, T, U, V
	Mouse	145-2C11	Novus Biologicals	NBP2-52641*
CD8+	Human	37006	R&D Systems	FAB1509-A, C, F, G, N, P, R, S, T, U, V
	Mouse	YTS 105.18	Novus Biologicals	NBP2-52659*
	Mouse	53-6.7	R&D Systems	FAB116-A, F, G, N, P, R, S, T, U, V
IFN-γ+	Human	25723	R&D Systems	IC285-A, C, F, G, N, P, R, S, T, U, V

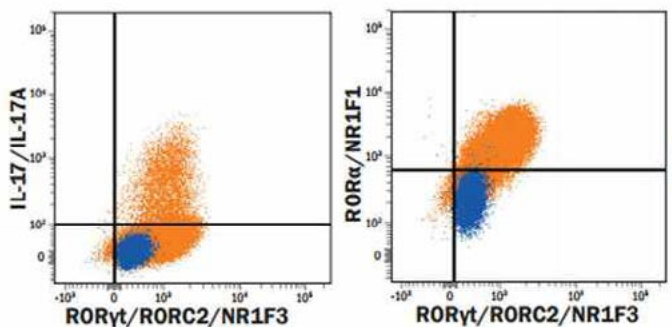

Figure 15. CD4+ T cells were isolated from total human PBMCs using a cell selection protocol, such as the one found in the MagCellect™ Human CD4+ T Cell Isolation Kit (Catalog # MAGH102). Isolated cells were incubated in media containing Recombinant Human IL-2 (Catalog # 202-IL), Human TGF-b1 (Catalog # 100-B), Recombinant Human IL-23 (Catalog # 1290-IL), Recombinant Human IL-6 (Catalog # 206-IL), Recombinant Human IL-1b (Catalog # 201-LB), and a Goat Anti-Human IFN-g Affinity-Purified Polyclonal Antibody (Catalog # AF-285-NA), followed by stimulation with PMA, calcium ionomycin, and monensin. Live, single, CD4+ cells are shown in the dot plots, determined using a fixable viability dye, doublet exclusion, and staining with an Alexa Fluor® 594-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791T). The cells were additionally stained using an Alexa Fluor® 700-conjugated Mouse Anti-Human IL-17/IL-17A Monoclonal Antibody (Catalog # IC3171N), a PE-conjugated Mouse Anti-Human RORa/NR1F1 Monoclonal Antibody (Catalog # IC8924P), and an Alexa Fluor® 488-conjugated Rabbit Anti-Human/Mouse RORgt/RORC2/NR1F3 Monoclonal Antibody (Catalog # IC9125G). Dot plots show the relative IL-17/IL-17A+, RORa/NR1F1+, and RORgt/RORC2/NR1F3+ cells in CD4+ resting (blue dots, lower left) and Th17-differentiated (orange dots, upper right quadrants) cell populations. Quadrant markers were set based on staining with the appropriate isotype controls (Catalog # IC003T, # IC0041N, # IC0041P, and # IC1051G). All cited reagents are from R&D Systems.

FLOW CYTOMETRY-VALIDATED ANTIBODIES				
CELL SURFACE MOLECULES	SPECIES	CLONE	BRAND	FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME)
CLASSICAL DENDRITIC CELLS				
CD11b/Integrin αM+	Human, Mouse, Rat +	Poly	Novus Biologicals	NB110-89474
	Human, Mouse +	M1/70.15	Novus Biologicals	NB600-1327
	Human	ICRF44	R&D Systems	FAB1699-G, N, R, S, T, U, V
	Mouse	M1/70	R&D Systems	FAB1124-A, F, G, N, P, R, , T, U, V
CD11c+	Human +	BU15	Novus Biologicals	NBP1-45018
	Human	3.9	Novus Biologicals	NB100-2711
	Human	ICRF3.9	R&D Systems	FAB1777-A, C, G, N, P, R, S, T, U, V
	Mouse	N418	R&D Systems	FAB69501-G, N, R, S, T, U, V
HLA-DR+	Human +	L243	Novus Biologicals	NB100-77855
	Human	TAL 1B5	Novus Biologicals	NB600-989
	Human	L203	R&D Systems	FAB4869-A, C, F, G, N, P, R, S, T, U, V
NATURAL KILLER CELLS				
CD3-	Human	UCHT1	R&D Systems	FAB100-A, C, F, G, N, P, R, S, T, U, V
	Mouse	17A2	R&D Systems	FAB4841-A, C, F, G, N, P, R, S, T, U, V
	Mouse	145-2C11	Novus Biologicals	NBP2-52641*
CD56/NCAM+	Human, Mouse, Rat	735	Novus Biologicals	NBP2-52669*
	Human, Rat +	123C3 (123C3. D5)	Novus Biologicals	NBP2-33132
	Human	ERIC-1	Novus Biologicals	NB100-2718
CD127/IL-7Rα-	Human	40131	R&D Systems	FAB306-A, G, N, P, R, S, T, U, V
	Mouse	1140A	R&D Systems	FAB7473-G, N, R, S, T, U, V *
	Mouse	A7R34	R&D Systems	FAB47742-A, G, N, P
T-bet/TBX21+	Human, Mouse +	4B10	Novus Biologicals	NBP1-43298
	Human	525831	R&D Systems	FAB53851-G, N, R, S, T, U, V *
EOMES+	Human	644730	R&D Systems	IC6166-A, G, N, R, S, T, U, V
	Mouse	1219A	R&D Systems	IC8889-G, N, P, R, S, T, U, V *

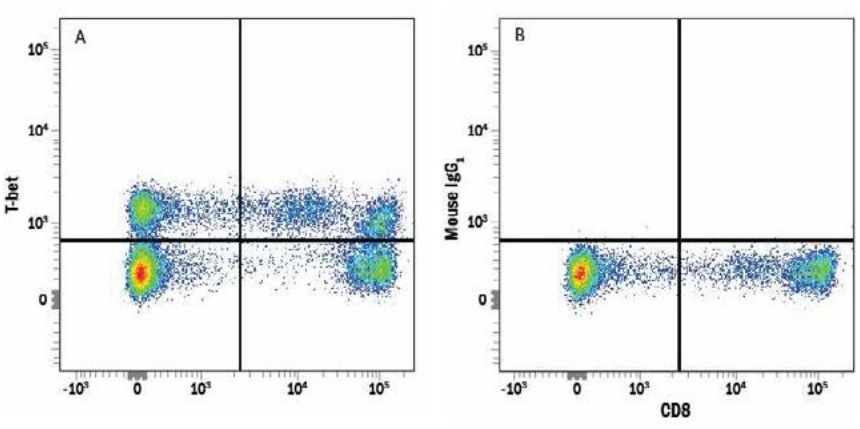

Figure 16. CD4+ Human peripheral blood mononuclear cells (PBMCs) treated with an anti-human IL-4 polyclonal antibody and Recombinant Human IL12 (Catalog # 219-IL) to induce Th1 cell development were stained with a PE-conjugated Mouse Anti-Human IFNg Monoclonal Antibody (Catalog # IC285P) and either an (**A**) Alexa Fluor® 488conjugated Mouse Anti-Human Tbet/TBX21 Monoclonal Antibody (Catalog # IC53851G) or a (**B**) Mouse IgG1 Alexa Fluor 488 Isotype Control (Catalog # IC002G). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). All cited reagents are from R&D Systems.

FLOW CYTOMETRY-VALIDATED ANTIBODIES				
CELL SURFACE MOLECULES	SPECIES	CLONE	BRAND	FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME)
M1 MACROPHAGES				
B7-1/CD80+	Human, Mouse	16-10A1	Novus Biologicals	NBP1-43385
B7-2/CD86+	Human, Mouse, Rat	BU63	Novus Biologicals	NBP2-25208
	Mouse	GL1	R&D Systems	FAB741-G, N, P, R, S, T, U, V
CD68/SR-D1+	Human, Mouse, Rat +	ED1	Novus Biologicals	NB600-985
	Human, Mouse, Rat	KP1	Novus Biologicals	NB100-683
	Human	298807	R&D Systems	IC20401-A, F, G, N, P, R, S, T, U, V
	Mouse	FA-11	Novus Biologicals	NBP2-33337
CD163-	Human +	EDHu-1	Novus Biologicals	NB110-40686
	Human, Rat	GHI/61	Novus Biologicals	NBP1-43341
	Human	215927	R&D Systems	FAB1607-A, C, G, N, R, S, T, U, V
	Rat	ED2	Novus Biologicals	NBP2-39099
HLA-DR+	Human +	L243	Novus Biologicals	NB100-77855
	Human	TAL 1B5	Novus Biologicals	NB600-989
	Human	L203	R&D Systems	FAB4869-A, C, F, G, N, P, R, S, T, U, V
iNOS+	Human, Mouse	4E5	Novus Biologicals	NBP2-22119
M2 MACROPHAGES				
CD163-	Human +	EDHu-1	Novus Biologicals	NB110-40686
	Human, Rat	GHI/61	Novus Biologicals	NBP1-43341
	Human	215927	R&D Systems	FAB1607-A, C, G, N, R, S, T, U, V
	Rat	ED2	Novus Biologicals	NBP2-39099
HLA-DRlow	Human +	L243	Novus Biologicals	NB100-77855
	Human	TAL 1B5	Novus Biologicals	NB600-989
	Human	L203	R&D Systems	FAB4869-A, C, F, G, N, P, R, S, T, U, V
MMR/CD206/Mannose Receptor+	Human	685641	R&D Systems	FAB25342-A, G, N, P, R, S, T, U, V

Fluorochrome Key: A Allophycocyanin, **C** PerCP, F Fluorescein, **G** Alexa Fluor® 488, **N** Alexa Fluor 700, **P** Phycoerythrin, **R** Alexa Fluor 647, **S** Alexa Fluor 750, **T** Alexa Fluor 594, **U** Alexa Fluor 350, **V** Alexa Fluor 405. For the full list of available fluorochromes, browse novusbio.com.

*Indicates a recombinant monoclonal antibody

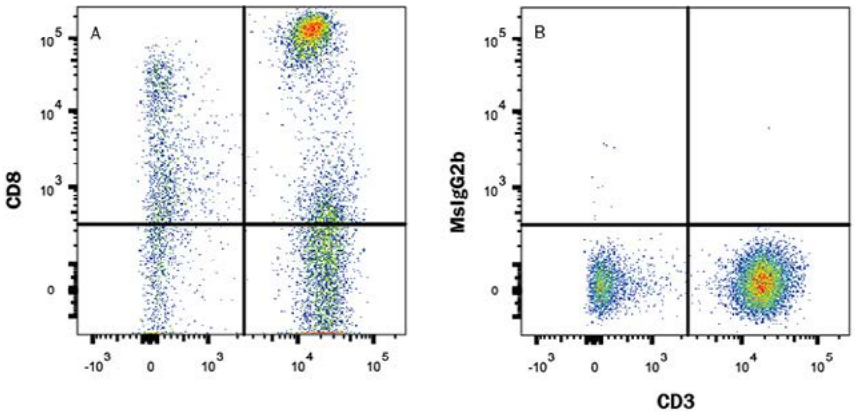

Figure 17. Human peripheral blood lymphocytes were stained with an (**A**) APC-conjugated Mouse Anti-Human CD8a Monoclonal Antibody (Catalog # FAB1509A) or a (**B**) Mouse IgG2B isotype control antibody (Catalog # IC0041A) and a PE-conjugated Mouse Anti-Human CD3e Monoclonal Antibody (Catalog # FAB100P). All cited reagents are from R&D Systems.

THERAPEUTICS RESEARCH AND DEVELOPMENT – STEM CELL RESEARCH

Mesenchymal Stem Cells (MSCs) are multipotent stem cells with the capacity to differentiate into various cell lineages. MSCs directly participate in the repair of tissue damage and modulate the immune response. MSCs have previously been reported to treat viral infections, specifically, alleviating acute lung injury caused by H5N1 and H9N2 influenza viruses. Production of chemokines and proinflammatory cytokines were reduced and MSCs limited the entry of inflammatory cells into the lungs. Thus, MSCs appear to suppress the adverse effects of the immune response and may relieve symptoms of viral infections by reducing inflammation, inflammatory exudations, and alveolar capillary endothelial cell loss.

While there are no clinically approved stem cell therapies to prevent and treat COVID-19 infections, several research projects have been initiated to investigate the use of MSC as a treatment option. Additionally, using lung organoids to simulate the pulmonary environment can provide a more accurate evaluation of drug efficacy. Bio-Techne offers workflow solutions for MSC and organoid research, including products for isolation, culture, differentiation and identification (Figure 18). We also offer a large supply of GMP cytokines and growth factors for ex vivo cell manufacturing. Full transparency and traceability of source and manufacturing systems is necessary for building a cell therapy product. As such, our large supply of GMP proteins is backed by our dedication to providing cell therapy manufacturers a consistent, safe, and traceable supply of reagents. Browse our full cell and gene therapy manufacturing portfolio at bio-techne.com.

PRODUCTS FOR MSCS

MSC ISOLATION KITS, CRYOPRESERVATION MEDIA & CONTAMINATION MONITORING

PRODUCT	BRAND	CATALOG #	DESCRIPTION
StemXVivo□ Serum-free MSC Freezing Media	R&D Systems	CCM016	For serum-free cryopreservation of Human/Mouse/Rat MSCs
CryoDefend□-Stem Cells Media	R&D Systems	CCM018	For defined MSC cryopreservation
Y-27632	Tocris	1254	Selective ROCK inhibitor; improves survival rate of stem cells undergoing cryopreservation
MagCellect□ Mouse MSC Isolation Kit	R&D Systems	MAGM212B	Uses negative selection to isolate mouse MSCs
MycoProbe Mycoplasma Detection Kit	R&D Systems	CUL001B	Detect common antibiotic-resistant cell culture contaminants

MSC EXPANSION MEDIA

PRODUCT	BRAND	CATALOG #	DESCRIPTION
StemXVivo□ Mesenchymal Stem Cell Expansion Media	R&D Systems	CCM004	Media for human, mouse, and rat MSCs
StemXVivo□ GMP Human MSC Expansion Media	R&D Systems	CCM026	GMP-grade media for human MSC expansion
StemXVivo□ Xeno-Free Human MSC Expansion Media	R&D Systems	CCM021	Free of non-human animal-derived components
StemXVivo□ Serum-Free Human MSC Expansion Media	R&D Systems	CCM014	Serum free expansion media

PROTEINS FOR MSC CULTURING

MOLECULE	BRAND	CATALOG #	GMP VERSION CATALOG #	SPECIES	SOURCE
BMP-2	R&D Systems	355-BM	355-GMP*	Human, Mouse, Rat	CHO
BMP-4	R&D Systems	314-BP	314-GMP	Human	NS0
EGF	R&D Systems	236-EG	236-GMP	Human	*E. coli*
FGF basic/FGF2/bFGF (146 aa)	R&D Systems	233-FB	233-GMP	Human	*E. coli*
IGF-I/IGF-1	R&D Systems	291-G1	291-GMP	Human	*E. coli*
PDGF-BB	R&D Systems	220-BB	220-GMP	Human	*E. coli*
TGF-β1	R&D Systems	240-B	240-GMP	Human	CHO
VEGF 165	R&D Systems	293-VE	293-GMP**	Human	*Sf* 21
Wnt-4	R&D Systems	6076-WN		Human	CHO
Wnt-5b	R&D Systems	7347-WN		Human	CHO
Wnt-10b	R&D Systems	7196-WN		Human	CHO

*The GMP version of BMP-2 is Human only.

**The source for the GMP version of VEGF 165 is *Sf* 9.

SMALL MOLECULES FOR MSC DIFFERENTIATION			
PRODUCT	BRAND	CATALOG #	DESCRIPTION
SP 600125	Tocris	1496	Selective JNK Inhibitor
SK 216	Tocris	6187	Plasminogen Activator Inhibitor-1 (PAI-1) inhibitor
Dexamethasone	Tocris	1126	Anti-inflammatory glucocorticoid
Zebularine	Tocris	2293	DNA methyltransferase and cytidine deaminase inhibitor
AICAR	Tocris	2840	AMPK activator
5-Azacytidine	Tocris	3842	DNA methyltransferase inhibitor
TCS 2210	Tocris	3877	Inducer of neuronal differentiation in MSCs
Nicotinamide	Tocris	4106	PARP-1 inhibitor
Kartogenin	Tocris	4513	Potently induces chondrogenesis in MSCs
Purmorphamine	Tocris	4551	Smo receptor agonist
Strontium chloride	Tocris	4749	Calcium Sensing Receptor (CaSR) agonist
GSA 10	Tocris	4918	Smo Receptor agonist
Liothyronine sodium	Tocris	5552	Thyroid Hormone (T3) analog; also promotes adipogenic differentiation of MSCs
KI-7	Tocris	6787	Positive allosteric modulator of A2B receptors

MSC IDENTIFICATION KITS, FLOW CYTOMETRY KITS & ANTIBODY PANELS				
PRODUCT	SPECIES	BRAND	CATALOG #	DESCRIPTION
MSC Functional Identification Kit	Human	R&D Systems	SC006	Verifies multipotency by *in vitro* functional differentiation
	Mouse	R&D Systems	SC010	
	Rat	R&D Systems	SC020	
MSC 4-Color Flow Kit	Human	R&D Systems	FMC002	Verifies MSC/stromal cell identity by flow cytometry
	Mouse	R&D Systems	FMC003	
MSC Verification Flow Kit	Human	R&D Systems	FMC020	Antibodies for MSC verification according to the markers proposed by the International Society for Cellular Therapy
MSC Marker Antibody Panel	Human	R&D Systems	SC017	Antibody panel for the verification of MSC/stromal cell identity by flow cytometry
	Mouse	R&D Systems	SC018	

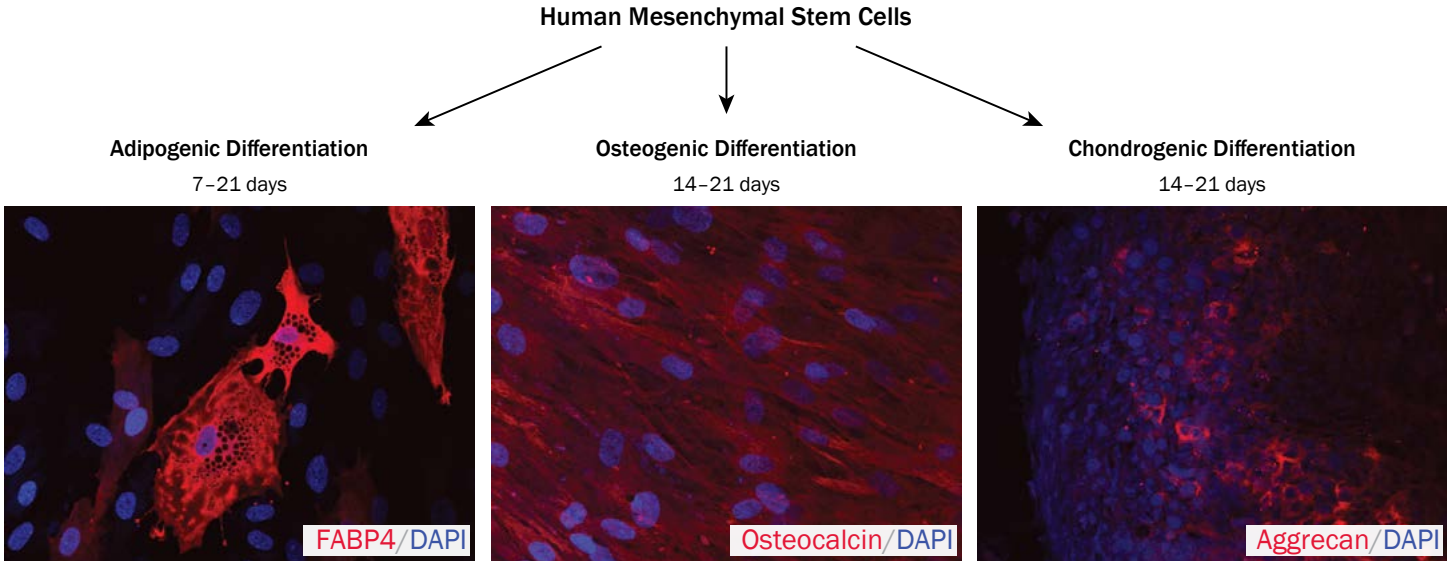

Figure 18. Human mesenchymal stem cells were cultured in StemXVivo™ Mesenchymal Stem Cell Expansion Media (Catalog # CCM004) and differentiation was induced as indicated using the media supplements included in the Human Mesenchymal Stem Cell Functional Identification Kit (Catalog # SC006). The kit also contains a Goat Anti-Mouse FABP-4 Antigen Affinity-Purified Polyclonal Antibody (adipocytes), a Goat Anti-Human Aggrecan Antigen Affinity-Purified Polyclonal Antibody (chondrocytes), and a Mouse Anti-Human Osteocalcin Monoclonal Antibody (Osteocytes) for the confirmation of differentiation status. The cells were stained using the NorthernLights™ 557-conjugated Donkey Anti-Goat (Catalog # NL001; red) or Anti-Mouse (Catalog # NL007; red) IgG Secondary Antibodies, and the nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

ANTIBODIES FOR MSC MARKERS						
MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
5′-Nucleotidase/ CD73+	Novus Biologicals	NBP1-85740	Human, Mouse, Rat +	Poly	ICC/IF, IHC, WB	No
	Novus Biologicals	NBP2-48480	Human	AD2	Flow, IHC	Yes
	R&D Systems	AF5795	Human	Poly	ICC/IF, IHC, SW, WB	No
	R&D Systems	AF4488	Mouse +	Poly	CyTOF, Flow, ICC/IF, IHC, WB	Yes
ALCAM/CD166+	R&D Systems	AF1172	Human, Mouse, Rat +	Poly	CyTOF, Flow, ICC/IF, IHC, SW, WB	Yes
	Novus Biologicals	NBP1-88129	Huma, Mouse, Rat	Poly	IHC	No
	R&D Systems	MAB6561	Human	105902	CyTOF, ELISA, Flow, WB	Yes
	R&D Systems	MAB1172	Mouse	200622	CyTOF, Flow, WB	Yes
CD11b-	Novus Biologicals	NB110-89474	Human, Mouse, Rat +	Poly	Flow, ICC/IF, IHC, ISH, ISH-IHC, SCW, SW, WB	Yes
	Novus Biologicals	NB600-1327	Human, Mouse +	M1/70.15	CyTOF, Flow, ICC/IF, IHC, IP	Yes
CD14-	Novus Biologicals	NBP2-37291	Human, Mouse	4B4F12	CyTOF, ELISA, Flow, ICC/IF, IHC, ISH-IHC, WB	No
	Novus Biologicals	NB100-77758	Human +	M5E2	B/N, CyTOF, Flow, ICC/IF, IHC	Yes
	R&D Systems	MAB3832	Human	134620	B/N, CyTOF, Flow	Yes
	R&D Systems	MAB982	Mouse	159010	CyTOF, Flow, WB	Yes
CD19-	Novus Biologicals	NBP2-24965	Human, Mouse, Rat	1D3	CyTOF, Flow, IV, IP	Yes
	Novus Biologicals	NBP2-25196	Human, Mouse	CB19	CyTOF, Flow, ICC/IF, IVT, WB	Yes
	R&D Systems	MAB4867	Human	4G7-2E3	CyTOF, Flow	Yes
CD34-	Novus Biologicals	NBP2-29455	Human, Rat	ICO-115	Flow, ICC/IF, WB	Yes
	R&D Systems	AF7227	Human	Poly	IHC, WB	No
	Novus Biologicals	NB600-1071	Mouse, Rat	MEC 14.7	ELISA, Flow, ICC/IF, IHC, IP, WB	Yes
	R&D Systems	AF4117	Rat	Poly	IHC, WB	Yes

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PRODUCTS FOR LUNG ORGANOIDS

BASE MEDIA FOR LUNG ORGANOID CULTURE			
PRODUCT	BRAND	CATALOG #	DESCRIPTION
N-2 MAX Media Supplement (100X)	R&D Systems	AR009	Fully defined supplement for culturing stem cells; alternative to N-2
GMP N-2 MAX Media Supplement (100X), Animal-free	R&D Systems	AR016	Serum-free and animal-free media supplement for *ex vivo* cell and tissue manufacturing under GMP-grade culture conditions
N-Acetylcysteine amide	Tocris	5619	Glutathione precursor and cell permeable antioxidant
Penicillin-Streptomycin 10/10 (100X)	R&D Systems	B21210	Contains 10,000 units/mL penicillin and 10,000 g/mL streptomycin
Ala-Gln	Tocris	5823	Stable form of L-glutamine
Organoid Harvesting Solution	R&D Systems	3700-100-01	Ready-to-use, non-enzymatic organoid harvesting and dissociation solution

3D GROWTH MATRIX COMPONENTS FOR LUNG ORGANOIDS					
PRODUCT/MOLECULE	BRAND	CATALOG #	GMP VERSION CATALOG #	SPECIES	SOURCE
Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2, Pathclear	R&D Systems	3533-010-02	N/A	N/A	N/A
Activin A	R&D Systems	338-AC	338-GMP	Human, Mouse, Rat	CHO
FGF basic/FGF2/bFGF (146 aa)	R&D Systems	233-FB	233-GMP	Human	*E. coli*
FGF-4	R&D Systems	235-F4		Human	*E. coli*
Noggin	R&D Systems	6057-NG	3344-GMP	Human	NS0
CHIR 99021	Tocris	4423	TB4423-GMP	N/A	N/A
SB 431542	Tocris	1614	TB1614-GMP	N/A	N/A

ANTIBODIES FOR LUNG ORGANOID MARKERS						
MOLECULE	BRAND	CATALOG #	SPECIES	CLONE	APPLICATIONS	CONJUGATES AVAILABLE
CKAP4/p63	Novus Biologicals	NBP1-26642	Human, Mouse	Poly	WB, IHC, IP	No
	R&D Systems	AF7355	Human	Poly	WB, ICC/IF	No
FoxJ1/HFH4	Novus Biologicals	NBP1-87928	Human, Mouse	Poly	IHC	No
	Novus Biologicals	NBP2-59032	Human, Mouse	CL3989	ICC/IF, IHC	No
	R&D Systems	MAB3619	Human	407003	CyTOF, Flow	Yes
	R&D Systems	AF3619	Human	Poly	ICC/IF, SW, WB	No
HOP	Novus Biologicals	NBP1-97503	Human, Mouse, Rat +	DS14F5	IHC, IP, WB	No
	Novus Biologicals	NBP1-92003	Human	Poly	IHC	No
ID2	Novus Biologicals	NBP1-88630	Human, Mouse, Rat	Poy	ICC/IF, IHC, WB	No
	Novus Biologicals	NBP2-66898	Human, Mouse	A4-D4	ICC/IF, IHC, WB	No
	Novus Biologicals	NBP2-27194	Human, Rat +	Poly	ICC/IF, WB	No
Lgr5/GPR49	Novus Biologicals	NLS1236	Human, Mouse, Rat +	Poly	ICC/IF, IHC	No
	Novus Biologicals	NBP1-28904	Human +	Poly	Flow, IHC, WB, ICC/IF (-)	Yes
	R&D Systems	MAB8078	Human	707042	CyTOF, FA, Flow, ICC/IF	Yes
	R&D Systems	MAB8240	Mouse	803420	CyTOF, FA, Flow, ICC/IF	Yes
Prosurfactant Protein C	Novus Biologicals	NBP2-37425	Human	5E6A9	ELISA, WB	No
	Novus Biologicals	NBP1-60117	Human	Poly	IHC, WB	No
α-Smooth Muscle Actin	Novus Biologicals	NBP2-33006	Human, Mouse, Rat +	1A4/asm-1	Flow, Flow-IC, ICC/IF, IHC, IP, SW, WB	Yes
	Novus Biologicals	NB300-978	Human, Mouse, Rat +	Poly	ICC/IF, IHC, PEP-ELISA, WB	No
TIF-1/NKX2-1	Novus Biologicals	NBP2-44501	Human, Mouse, Rat	8G7G3/1+NX2.1/690	Flow, ICC/IF, IHC	Yes
	Novus Biologicals	NBP2-32999	Human, Mouse, Rat	SPM150	Flow, ICC/IF, IHC, IP, WB	Yes
	Novus Biologicals	NBP2-41160	Human, Mouse, Rat	Poly	ELISA, ICC/IF, IHC, WB	No
Uteroglobin/SCGB1A1	Novus Biologicals	NBP2-75705	Human, Mouse, Rat	JU34-03	Flow, ICC/IF, WB	No
	R&D Systems	MAB4218	Human	394324	IHC, WB	No
	R&D Systems	AF4218	Human	Poly	B/N, IHC	No

Species Key: + Additional Species Available

Applications Key: B/N Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **FA** Functional Assay, **Flow** Flow Cytometry, **Flow-IC** Flow Cytometry (Intracellular), **IB** Immunoblotting, **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **ISH** *In Situ* Hybridization, **ISH-IHC** Dual ISH-IHC, **IV** *In Vivo*, **IVT** *In Vitro*, **KO** Knockout Validated, **PEP-ELISA** Peptide ELISA, **SCW** Single Cell Western, **SW** Simple Western™, **WB** Western blot

THERAPEUTICS RESEARCH AND DEVELOPMENT – VACCINE DEVELOPMENT

TcBUSTER™: FAST AND FLEXIBLE STABLE CELL LINE DEVELOPMENT FOR VACCINE PRODUCTION

Development of a SARS-CoV-2 vaccine is the most effective way to prevent future infections. The urgency for such a remedy is driving research into available approaches for vaccine development. Stable expression cell-based systems can offer a faster and more reliable strategy for vaccine production. Novel production systems using mammalian or insect cell cultures will be needed to overcome the limitations of current stable cell line systems in order to produce large amounts of vaccine as quickly as possible.

TcBuster™, the transposon gene-editing system from Bio-Techne's B-MoGen brand, can accelerate the development and production of your protein subunit or virus-like particle vaccine by giving you a stable cell population in as little as three weeks, and clonal cultures in six weeks. The generation of stable over-expressing cell lines using our system offers several advantages:

- A faster, stable cell line generation than when using virus-mediated, CRISPR and plasmid systems alone
- Stable integration of multiple transgenes in one transfection
- Higher protein-producing clones compared to stable cells created by site-specific integration
- A high integration rate that facilitates identification of a high producing clone
- Sizable protein yields produced from mammalian, chicken and insect cell lines

STABLE CELL LINES FOR VIRUS-LIKE PARTICLE (VLP) VACCINES

- Separate transgenes on multiple transposons can create higher expression compared to polycistronic signal.
- Mammalian cells more accurately produce glycosylated VLPs and recombinant subunits. TcBuster can quickly create stable cell lines in a number of different cell types that have different glycosylation patterns.
- A vast diversity of clones can be created from a single transfection.
- High efficiency of random integration creates a higher likelihood of finding high producing clones that have favorable chromosome-position effects
- TcBuster stable cell line production could replace baculovirus, thereby decreasing the purification need and difficulty. Additionally, baculovirus may mask immune responses against the desired epitope.
- Stable cell line generation with TcBuster can also be used in tandem with baculovirus to create a system that produces VLP at a higher order of magnitude.

STABLE CELL LINES FOR SUBUNIT VACCINES

- Glycosylation patterns play an enormous role in the immunogenicity of subunit vaccines. Stable expression of transgenes produces more accurate and consistent glycosylation than with transient expression.
- Alternate or aberrant glycosylation patterns can also increase the immunogenicity of a subunit vaccine. Cell lines can be altered to overexpress glycosylation enzymes that shift the glycosylation profile of a cell. In addition to moving in your vaccine subunit, we can introduce enzymes that alter its glycosylation pattern all in one transfection.

INSTRUMENTATION FOR VACCINE DEVELOPMENT AND PRODUCTION

From early stage research to vaccine release, Bio-Techne offers innovative and market-leading technologies to support the development and quality control of a COVID-19 vaccine (Figure 19). The pioneering protein analysis solutions from our ProteinSimple brand have already shaped viral-based disease research progress. Our instruments have supported research aimed at developing a new inactivated poliovirus vaccine (Thomassen *et al.*, 2013), detecting norovirus particles particles through capillary isoelectric focusing-whole column imaging detection (CIEF-WCID) (Goodridge *et al.*, 2004), elucidating mechanisms of MERS pathogenesis (Gassen *et al.*, 2019), and developing an identity assay for a 15-valent pneumococcal conjugate vaccine (Hamm *et al.*, 2015). The COVID-19 pandemic presents a dynamic and evolving threat to public health, challenging researchers to quickly understand SARS-CoV-2's pathogenic mechanisms, identify protein targets key to its survival and infectivity, and to develop effective treatments to stop it.

	SINGLE CELL WESTERN	SIMPLE WESTERN™	MICRO-FLOW IMAGING	IcIEF AND CE-SDS	SIMPLE PLEX™
Cell Line Development	√	√	√		√
Strain Selection	√	√			
Analytical Development		√	√	√	√
Cell Bank Development	√				
Upstream Process Development		√	√		√
GLP Substance Production			√		
Formulation Development		√	√	√	√
Process Characterization			√	√	
Validation/Documentation				√	
GMP Batch Production				√	
Stability Studies		√	√	√	

Figure 19. Bio-Techne's analytical solutions from its ProteinSimple brand that are key for facilitating vaccine development.

DISCERNING CELL TO CELL VARIABILITY WITH SINGLE CELL WESTERN

A first step in vaccine development is the growth and expansion of cell lines producing candidate viral antigens. Knowing the clonality of the cell line is key and Single-Cell Westerns (scWs) facilitate the detection of cell-to-cell variation. Milo™, Bio-Techne's scW platform, generates robust Western-based protein expression data while measuring target expression heterogeneity and cell type heterogeneity (Figure 20). The fast, automated scW workflow enables analysis of ~1,000 single cells per run, multiplexing up to four proteins per cell, and uses conventional Western blot antibodies.

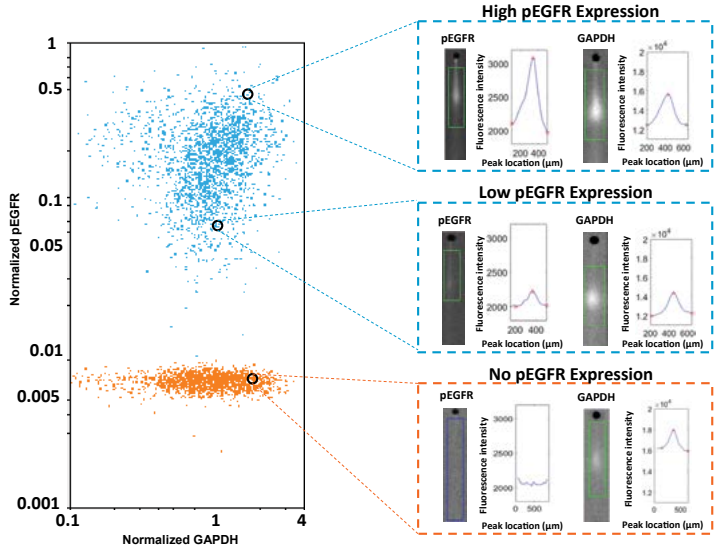


Figure 20. EGFR expression and phosphorylation were analyzed by scW. Positive pEGFR populations (blue) are easily separated from negative pEGFR populations (orange). The cluster of pEGFR positive cells can be further sub-divided into high/low pairing.

ELUCIDATING THE MECHANISM OF VIRAL INFECTIONS BY SIMPLE WESTERN™ ANALYSIS: A MERS-COV CASE STUDY

In studies to uncover the mechanism of MERS infection, Gassen *et al.* (2019) showed that MERS-CoV benefits from reducing autophagy of its host by blocking AP-lysosome fusion. They used Simple Western analysis to show that MERS-CoV infection significantly reduced ATG14 oligomerization (Figure 21), which plays an important role in autophagic activity by promoting autophagosome fusion to the lysosome. They also showed that ATG14 oligomers were enhanced following inhibition of the S-phase Kinase-associated Protein 2 (SKP2) E3 ligase (Figure 22). Quantitative analysis using Simple Western assays showed an approximate 2.5 fold increase in ATG14 oligomers (Figure 22). These results suggest that SKP2 inhibition promotes autophagy and reduces MERS-CoV infection.

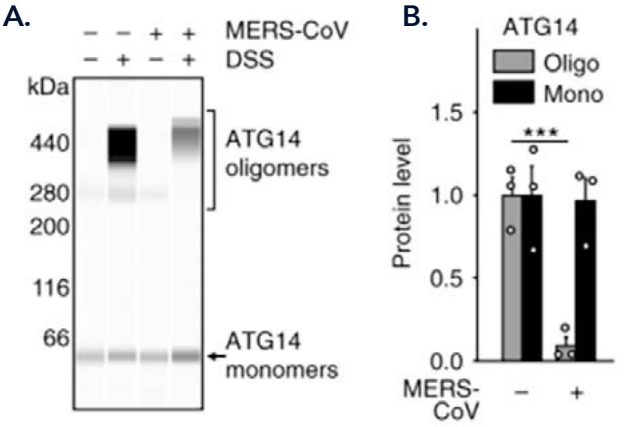


Figure 21. VeroB4 cells were infected with MERS-CoV (MOI= 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 minutes and harvested. ATG14 homo-oligomerization was examined with Simple Western (A) and quantified (B). ****p<0.001. Image adapted from Gassen et al. (2019) Nat. Commun.* ***10****:5770.*

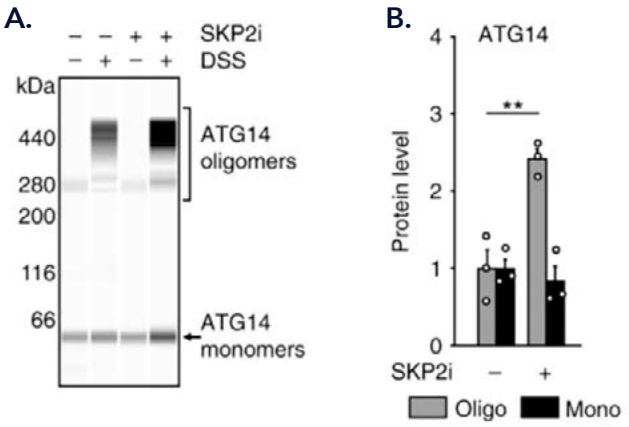
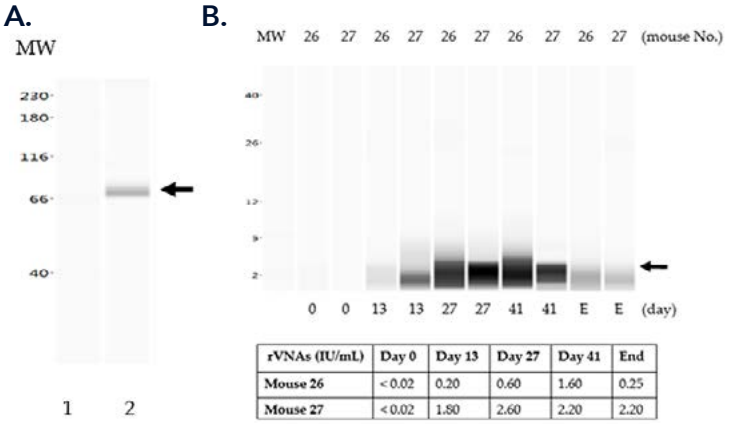


Figure 22. VeroB4 cells were infected with MERS-CoV (MOI= 0.001), treated with SKP2i for 48 h, cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 minutes and harvested. ATG14 homo-oligomerization was assessed with Simple Western (A) and quantified (B). ***p<0.01. Image adapted from Gassen et al. (2019) Nat. Commun.* ***10****:5770.*

Simple Western is the first fully automated and complete solution for protein detection and characterization, removing the manual, error-prone steps in standard Western blots. In Simple Western assays, proteins can be separated by charge or size from 2 – 440 kDa and detected in as little as 3 hours. Protein is quantified by immunoprobing or total protein labeling with the ability to process up to 96 samples at once. Bio-Techne also offers over 1,300 primary antibodies from its R&D Systems and Novus Biologicals brands that are validated for Simple Western.

DETECTION AND CHARACTERIZATION OF HOST RESPONSES TO VACCINES WITH SIMPLE WESTERN™ ANALYSIS

Tracking the impact of a vaccine on a recipient is critical for understanding and improving vaccine effectiveness. Wu *et al*. (2019) developed a vaccine against rabies (ERA-2GnRH) that contains a sterilizing agent (GnRH) to control animal populations. They used Simple Western to track the immune response following vaccine administration in mouse models. They observed the rise and fall in antibody levels against GnRH, which corresponded with the rabies viral neutralizing antibodies (rVNAs) (Figure 23). They concluded that there was a concurrent immune response between the GnRH antigen and its vector RABV.



rVNAs (IU/mL)	Day 0	Day 13	Day 27	Day 41	End
Mouse 26	< 0.02	0.20	0.60	1.60	0.25
Mouse 27	< 0.02	1.80	2.60	2.20	2.20

Figure 23. Simple Western was used to detect recombinant protein G-2GnRH (A, lane 1) and recombinant G-2GnRH protein from purified ERA-2GnRH virus (A, lane 2). GnRH antibodies were detected in mice at various times points after vaccination (B). *Image adapted from Wu et al. (2019) Vaccines* ***7****:73.*

CHARACTERIZATION AND DETECTION OF VACCINE IMPURITIES WITH SIMPLE WESTERN™

Bovine serum albumin (BSA), is often an important gauge for quality control in vaccine manufacturing The WHO sets limits on the level of residual impurities allowed in vaccines, including residual BSA. When analyzing BSA levels, it is important to separate the monomeric form from BSA aggregates or degraded products, both of which are indistinguishable from the monomer by ELISA. While SDS-PAGE provides a relatively easy way to measure BSA, Loughney *et al*. (2014) found that a key viral protein antigen for their vaccine migrated at the same molecular weight as BSA. With Simple Western, the Vaccine Analytical Development team at Merck developed a fast and sensitive assay to specifically monitor BSA levels throughout vaccine production and in the final product.

IMAGED cIEF: A CRITICAL TOOL FOR VACCINE DEVELOPMENT AND MONITORING VACCINE STABILITY

Biopharmaceuticals are often large molecules with complex structures carrying various post-translational modifications (e.g., oxidation, glycosylation, glycation, and deamination) that may lead to changes in their charge distribution. Charge heterogeneity analysis is required for the batch release of biologics as it can be altered by stress, manufacturing changes, age, or other factors. Because charge variants may have different tissue distribution and pharmacokinetics, their characterization is a production quality requirement ensuring consistent biological activity. Imaged capillary isoelectric focusing (icIEF) is the industry gold-standard for charge variant analysis of biopharmaceuticals. Many researchers perform icIEF assays using Bio-Techne's ProteinSimple brand iCE3 or Maurice systems for the development and manufacture of therapeutic monoclonal antibodies, and for characterizing viruses and vaccines.

Merck leveraged the icIEF platforms, iCE3 and Maurice, to study vaccine stability and identity. They used Maurice to analyze lipid nanoparticles (LNPs), which have recently shown great promise as efficient drug delivery systems, by icIEF (Figures 24-25). In this platform, proteins are measured directly with absorbance or using native fluorescence. The observed that LNPs with different cationic lipids have unique pIs (Figure 24). They also saw that increasing the temperature shifted the pIs to lower values and produced a new peak, which was analogous to acidic variants (Figure 25). Characterization of these charge profiles by cIEF revealed critical differences that may have been missed by size-based separation approaches.

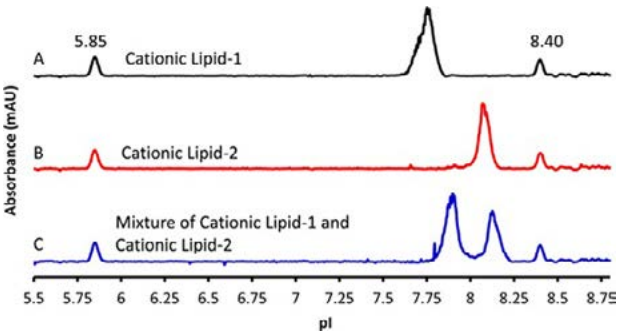


Figure 24. icIEF analysis of LNPs with Bio-Techne's ProteinSimple brand Maurice show different cationic lipids have unique pIs. *Image from Loughney, J.W. et al. (2019) Electrophoresis* ***40****:2602.*

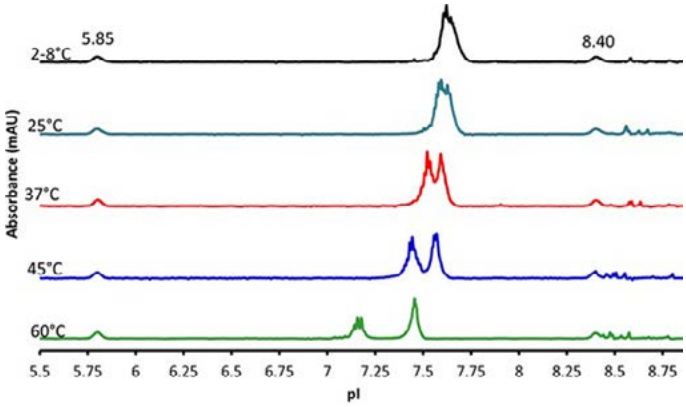


Figure 25. LNPs containing mRNA that were stored under elevated temperatures were analyzed with cIEF. The LNP stored at 2–8°C showed a symmetrical peak shape with a pI of approximately 7.7. As the temperature increased, the LNP with mRNA peaks became more acidic and split into two distinct peaks. *Image from Loughney, J.W. et al. (2019) Electrophoresis* ***40****:2602.*

CHARACTERIZING VACCINE PARTICULATES WITH MICRO-FLOW IMAGING (MFI)

Sub-visible particulate contaminants are a cause for concern in pharmaceutical products. These contaminants, either from an intrinsic or extrinsic source, may include glass, silicon oil, rubber, and product aggregates. These particulates impact safety and efficacy of products, requiring continuous monitoring throughout production and in the final products. Similar to other biopharmaceuticals, vaccine candidates are often put through forced degradation studies to support analytical method development, obtain information on degradation, and identify optimal conditions and potential stabilizers for long-term storage. Whitaker *et al.* used MFI to assess the aggregation propensities and overall stabilities of trimers of the HIV-1 candidate vaccines, GT1.1 and SOSIP.664 gp140, during agitation (Figure 26). They found these parameters to be comparable when both trimers were formulated in the same buffer.

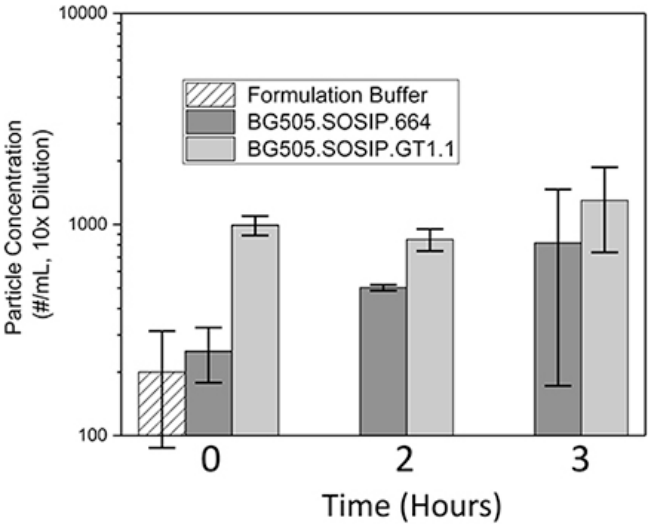


Figure 26. Subvisible (2-100 mm) particle formation (# of particle/mL) during agitation stress studies of BG505 SOSIP.664 and GT1.1 gp140 trimers as determined by MFI. *Graph from Whitaker, N. et al. (2019) J. Pharm. Sci.* ***108****:2264.*

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