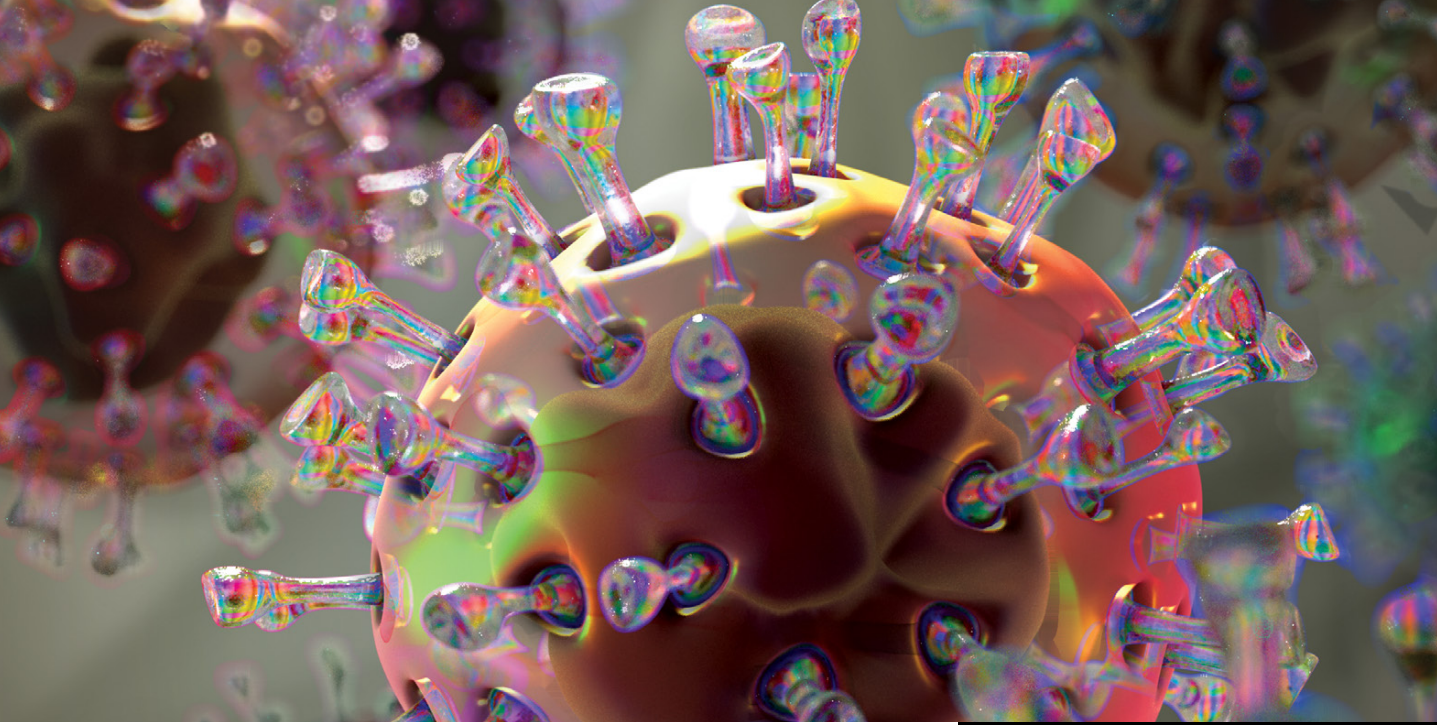


# Accelerating Vaccines and Virus Research

**Live Cell Analysis, Advanced Flow Cytometry and Label-Free Binding Analysis**

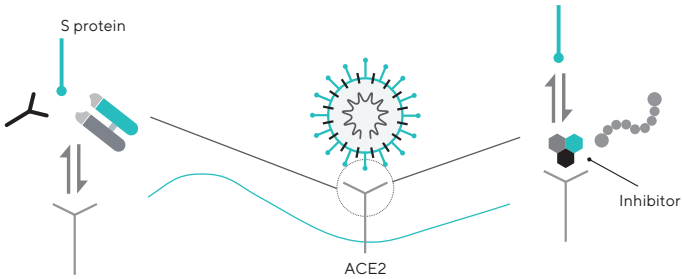
Simplifying Progress

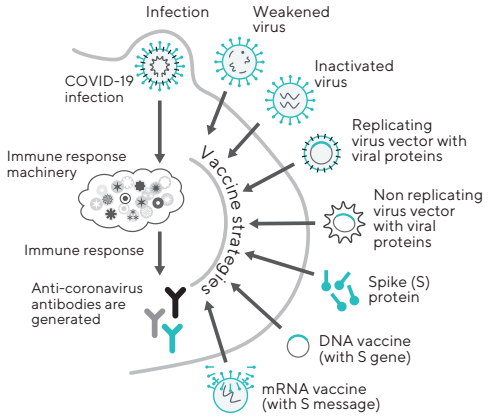
SARTORIUS

# Introduction

Vaccines research, development and manufacturing processes saw unprecedented advancements over the past two years while responding to the global COVID-19 pandemic largely due to the efforts and collaborations in the scientific community. Vaccine development is a multi-step process that begins with the analysis of virus biology, infection mechanisms, and host responses before determining vaccine and other therepeutic mechanisms. These are often followed by candidate selection, pre-clinical and clinical assessment before regulatory approvals for use in humans. While highly effective COVID-19 vaccines were developed and approved for public use less than an year since the SARS-CoV 2 genomic sequence was published, this also prompts us of the constant need for continued development of vaccine research and manufacturing processes to combat future viral threats at even faster timelines.

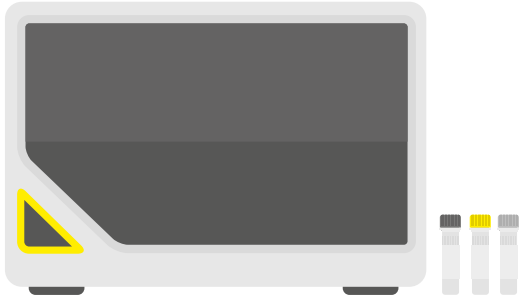
See how Sartorius' innovative solutions advance and accelerate discovery and development of new vaccines and therapeutics against viral infections.



# Incucyte® Real-Time Live-Cell Analysis

The Incucyte® Live-Cell Analysis Systems can automatically capture and analyze images from multiple microwell plates in parallel, thereby significantly increasing throughput. A unique moving optical path design allows cells and cell plates to remain stationary throughout the entire experiment which further minimizes cell perturbance and enables imaging and analysis of both adherent and suspension cell types. Incucyte® enables acquisition and analysis of data throughout the course of an experiment to capture time dependent effects compared endpoint analysis by traditional cell culture analysis techniques.



**Additional Information (click to expand)**

- Features and Benefits +
- Key Advantages +
- Featured Assays +

- Real-time continuous kinetic measurements
- Never miss a data point
- Networked remote access. Minimizes risk of expoure
- Monitor viral infection, cell health, movement, morphology and functional response

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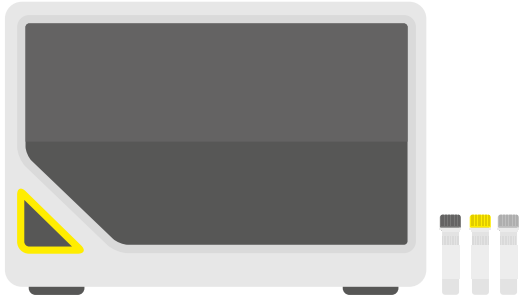


**Additional Information (click to expand)**

- Features and Benefits
- Key Advantages
- Featured Assays
  1. Real-time comparisons of virus infection kinetics and efficiencies
  2. Kinetic analysis of NETosis
  3. Measuring the effect of VSV aggregate infectious units

- Real-time continuous kinetic measurements
- Never miss a data point
- Networked remote access. Minimizes risk of expoure
- Monitor viral infection, cell health, movement, morphology and functional response

# Incucyte® Real-Time Live-Cell Analysis

## Features and Benefits

| Feature | Benefit |
|---|---|
| Up to five different fluorescence channels, up to three at a time in a single experiment | Do more, specifically design for live-cell analysis |
| New 3-color optical module includes a long wavelength, low phototoxicity NIR channel and optimized reagent for turn-key applications | More applications. Enable multiplexed, kinetic analysis in living cells |
| Supports 3 interchangeable vessel trays and over 600 vessels, up to 6 microplates in parallel | Redice artifacts with a consistent, physiologically-relevant environment during the entire experiment |
| New applications available to monitor metabolism, neuronal activity, and organoids | Increased capacity |
| Seamless multi-user support via remote, networked access and unlimited, free licenses | Improve productivity and flexibility |

# Incucyte® Real-Time Live-Cell Analysis

## Key Advantages

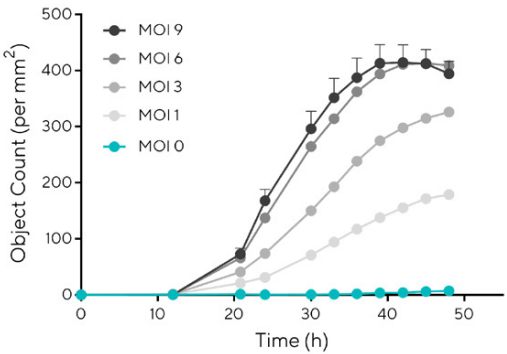
- Quantify cell-specific phenotypic changes in expression markers or morphology within mixed cultures in an unperturbed micro-environment
- Measure fractional changes in number of cells labeled with your cell surface protein of interest
- Reveal drug mechanisms of action based on subpopulation studies of cell cycle phase or cell health
- Easily generate kinetic data from thousands of images using unique Cell-by-Cell image analysis tools and live-cell reagents
- Study functional kinetic data from identified cellular subsets and compare to total population data

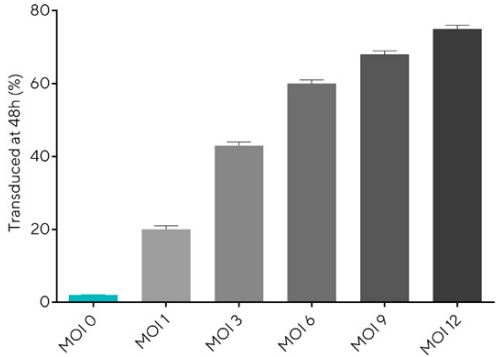
**Learn More**

# Incucyte® Real-Time Live-Cell Analysis

## Featured Assays

**1. Continuously monitoring virus infections by live-cell analysis enables real-time comparisons of virus infection kinetics and efficiencies**



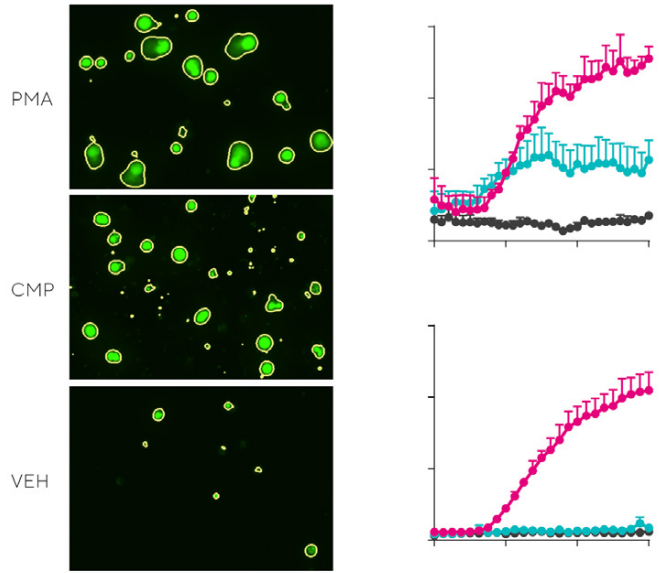

Time-course of nuclear GFP expression in A549 human lung carcinoma cells transduced with Incucyte® Nuclight Green Lentivirus. Transduction of cells with Nuclight Green is titratable in HUVECs.

# Incucyte® Real-Time Live-Cell Analysis

## Featured Assays

**2. Kinetic analysis of NETosis**

Neutrophils are the first line of defense at the site of an infection, playing an essential role in the innate immune system, employing multiple strategies to degrade and kill microbes. One of these strategies is programmed neutrophil cell death known as NETosis, during which neutrophils corner and destroy the invading pathogens and abnormal cells through the formation of neutrophil extracellular traps (NETs). Incucyte® live-cell analysis of NETosis addresses many of the challenges imposed by standard end-point methods by enabling real-time, automated, direct visualization, and quantification of NETosis throughout the time-course of NET formation and release.



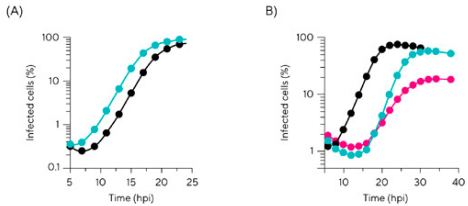

Quantification of NETs by fluorescence masking with Incucyte® Live-Cell Analysis System. NET formation following PMA, CMP or vehicle treatment is visualized using the Incucyte® Cytotox Green Dye. Kinetic time course graphs of Average Green Area and Total Green Area allow for separation of cells undergoing NETosis (PMA-stimulated) and cells undergoing apoptosis (CMP-treated). Incycyte integrated software and fluorescent masking algorithms (yellow outline) yielded average fluorescent object size (top right graph) which clearly separated NETosing (PMA-stimulated) and apoptotic (CMP treated) cells. Additionally, using a size exclusion filter to remove smaller objects, such as dead cells, from the analysis allows for direct quantification of the NETosis signal (bottom right graph).

# Incucyte® Real-Time Live-Cell Analysis

## Featured Assays

**3.Measuring the effect of VSV aggregate infectious units on infectivity rates by live-cell analysis**

Certain viruses can infect cells from multi-virion structures called collective infectious units. Andreu-Moreno and Sanjua et al. showed that collective infection by aggregates of vesicular stomatitis virus (VSV) accelerates viral progeny production and increases short-term fitness compared to free viruses, revealing a cellular-level Allee effect. Authors investigated this effect by studying viral fitness of free VSV particles verses those that were aggregated in saliva (1). This study leveraged the kinetic capabilities of real-time, live-cell imaging and analysis with Incucyte® to compare fitness effects of viral particles dispersed in aggregated form to those in monodisperse. Real-time, live cell kinetic studies were used to study mouse embryonic fibroblasts (MEFs) inoculated with either monodispersed VSV-mCherry particles or aggregated VSV-GFP using human saliva. The growth curves, generated from the Incucyte® analysis, revealed leftward shift for viral populations formed by aggregates, as compared to those which were monodispersed. Aggregation of virus particles imparted a short-term fitness advantage and increased the release of viral progeny, which was dependent on cellular permissively to infection and was correlated with the level of cellular innate immunity.course of NET formation and release.

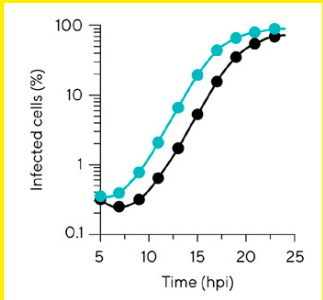



Click for graph detail

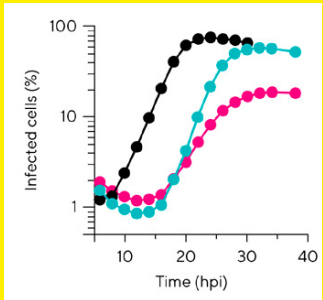
# Incucyte® Real-Time Live-Cell Analysis

## Featured Assays - Graph Detail

A




B




**Real-time fluorescent measurements to determine VSV infection Incucyte®.**

**(A)** Growth curves of VSV in Mouse Embryonic Fibroblasts (MEF) obtained by real-time whole-well fluorescence cell analysis. The percentage of fluorescent cells in the well is shown. Monodisperse inoculum (black) and aggregated inoculum (teal). Real-time fluorescence microscopy revealed that the growth curves of viral populations founded by aggregates were left shifted compared to those founded by monodisperse particles, with estimated half-times of 16.7 ± 0.2 hr and 19.1 ± 0.1 hr, respectively.

**(B)** The fitness benefit of aggregation Is lost in mutagenized populations. Growth curves of VSV in MEFs by real-time whole-well fluorescence live-cell analysis. Percentage of fluorescent cells in the wells are shown. Non-mutagenized monodisperse inoculum (gray), mutagenized monodisperse inoculum (red), mutagenized aggregated inoculum (red). (gray), mutagenized monodisperse inoculum (red), mutagenized aggregated inoculum (red).

**Click to close detail**

# iQue® Advanced Flow Cytometry

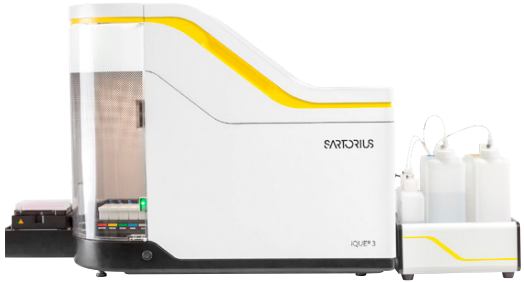
The advanced iQue® flow cytometry platform enables high throughput analysis of immune cell profiling and functional assessment, immune cell activation and antibody screening with minimal sample volume and rapid results, in real time and without compromising your cell cultures.

Ready-to-use kits including the iQue® T Cell Phenotyping kit, Human iQue Qbeads® Inflammation Panel for cytokine storm assessment, and kits for T cell activation, memory, killing and exhaustion along with cytokine profiling enable greater in depth characterization of the host immune response in each sample. The timesaving, user-friendly iQue Forecyt® software offers plate-level annotation, analytics, and results visualization tools ideally suited for screening large data sets. Data from wells can be linked together and multiple assay outcomes combined to facilitate "hit" identification.

**Additional Information (click to expand)**

Key Advantages +

Featured Assays +




- High-throughput multiplexed cytometry screening and real time data analysis
- Sample as little as 1 ul from minituarized assay volumes
- Multiplex cell phenotypes and cytokines in the same well

# iQue® Advanced Flow Cytometry

## Key Advantages

- **Speed** — Fastest plate sampling, integrated analysis, multiplexed no-wash assays, and novel data reduction tools
- **Miniaturization** — Lowest assay volumes and sample volumes as little as a single microliter saves reagents and conserves precious cells
- **Content** — High content, multiplexed analysis of cells, beads and secreted proteins
- **Usability** — Simple, scalable, multi-user environment with walkaway automation, comprehensive analysis and visualization tools
- **Insight** — Link information, run scenarios, create knowledge, make better decisions

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- Multiplex cell phenotypes and cytokines in the same well

**Additional Information (click to expand)**

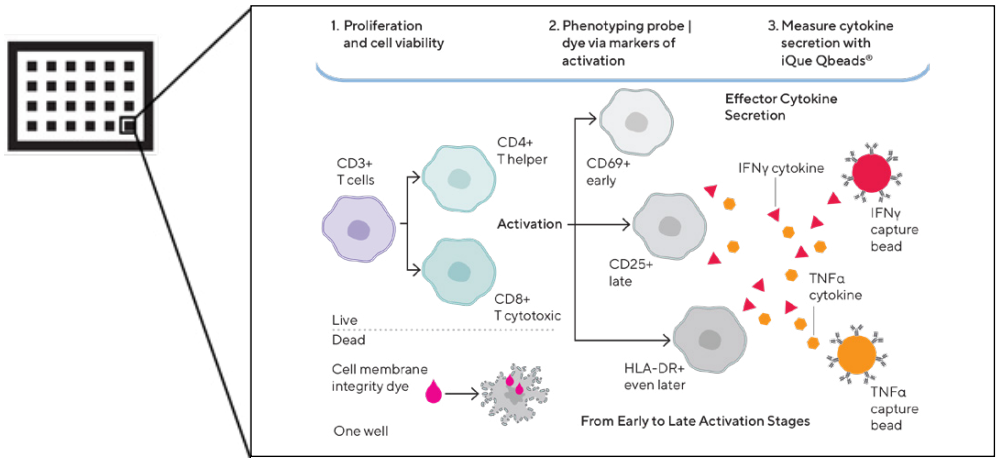
- Key Advantages +
- Featured Assays ×
  1. Combined phenotype and function assays including iQue Qbeads® Kits +
  2. Antibody Characterization +
  3. Multiplex assay to simultaniously measure antibodies to different SARS-CoV-2 proteins and isotypes +

# iQue® Advanced Flow Cytometry

## Featured Assays

**1. Combined phenotype and function assays including iQue Qbeads® Kits**

The iQue Qbeads® family of reagents enables capture of specific proteins on distinct beads to facilitate multiplexed quantitation of a range of proteins, cytokines, enzymes, and growth factors etc. in a single well at microliter assay volumes. An all-in-one assay format to assess immunophenotype, cytokine profiles, and cell health in the same well using a tenth of the sample volume compared to traditional flow cytometry assays.



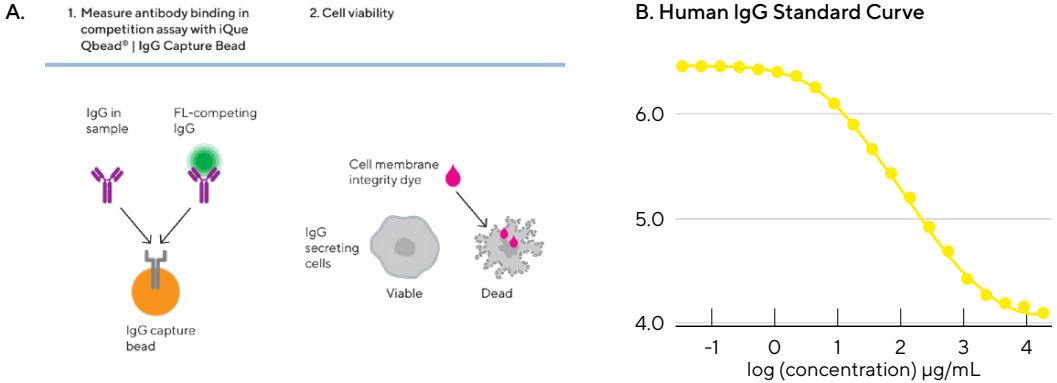

The iQue® Human T Cell Activation Kit streamlines the traditional workflow by measuring immune cell phenotypes, T cell activation markers, cell proliferation, cell viability and secreted cytokine concentrations (IFNγ and TNFα) using only 5 µL–10 µL of samples

# iQue® Advanced Flow Cytometry

## Featured Assays

**2. Antibody Characterization - iQue® Human IgG Titer and Viability Kit**

The iQue® Human IgG Titer and Viability Kit enables the rapid analysis of thousands of clones in a simple no-wash, mix and read assay. It is the only solution that allows correlation of IgG quantitation, cell viability and cell count in a single well to make more informed decisions on cell productivity.




A) Principle of the iQue® Human IgG Titer and Viability Kit. Fluorescently labeled IgG (FITC-IgG) is added to samples containing secreted IgG and CHO production cells. The FITC-IgG and non-labelled sample IgG compete for binding to IgG capture beads. Cell viability is simultaneously measured in each well using a membrane impermeable integrity dye.

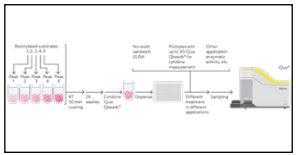
(B) IgG concentration is inversely proportional to intensity of fluorescence signal.

# iQue® Advanced Flow Cytometry

## Featured Assays

**3. Multiplex assay to simultaniously measure antibodies to different SARS-CoV-2 proteins and different isotypes**

Dogan et al. (2021) developed a highly sensitive bead-based flow cytometry assay. Since this assay can be multiplexed, antibodies to different SARS-CoV-2 proteins and different isotypes can be measured simultaneously [2]. To access antibody neutralization, the team developed a pseudotyped SARS-CoV-2 and SARS-CoV infection assay. These assays were tested using samples from normal donors and the following SARS-CoV-2 positive groups: outpatient, hospitalized, and ICU diseased patients. The iQue Qbeads® DevScreen SAv bead kit was used to bind biotinylated spike protein RBD, nucleocapsid protein, S1 protein, and other proteins to beads for use in fluorescent assays. The iQue® platform was used to measure binding of antibodies to beads labeled with different SARS-CoV-2 proteins. iQue Qbeads® DevScreen SAv beads were gated using FSC-H/SSC-H, and singlet beads gate was created using FSC-A/FSC-H. Gates for different iQue Qbeads® DevScreen SAv beads were determined based on their fluorescence signature on RL1-H/RL2-H plot (on iQue® platform). PE fluorescence median, which is directly associated with each single plex beads, was determined using BL2-H (on iQue® platform).



Click to expand

iQue Qbeads® DevScreen SAv, part of the Qbeads® DevScreen family, are bead-based kits that provide you more flexibility to make your own bead-based multiplex assays. iQue Qbeads® DevScreen SAv are streptavidin coated beads that can be used to screen with biotinylated targets. There are 5 different SAv-coated bead populations, and these can be multiplexed.

# iQue® Advanced Flow Cytometry

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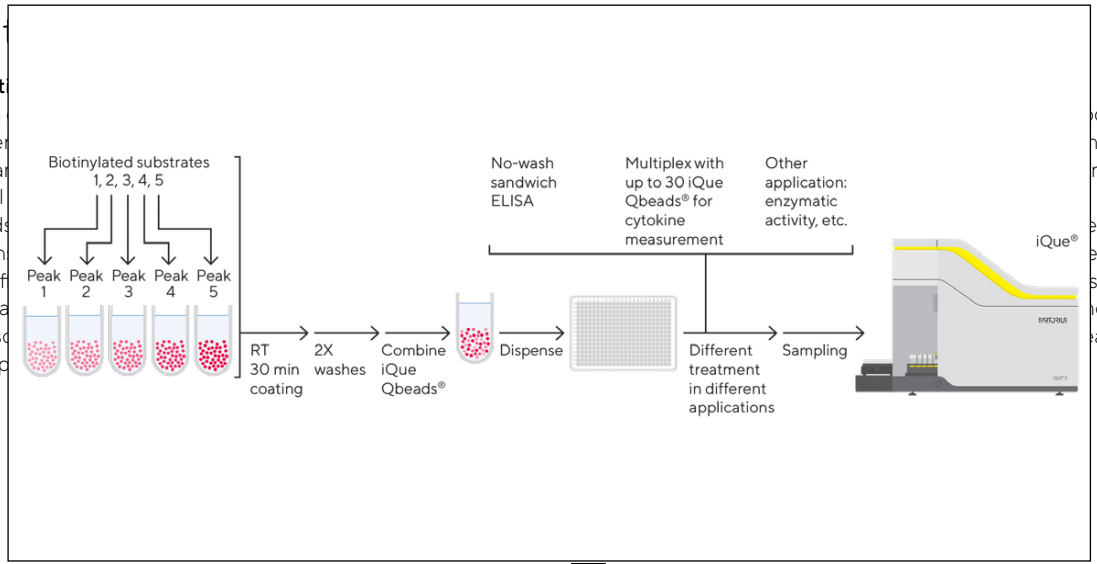


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# Octet® Bio-Layer Interferometry

Octet® BLI label-free binding and concentration measurement assays are widely deployed in vaccine and therapeutics development programs due to its ability to rapidly provide binding information critical to the lead selection process. Traditional methods for analyzing biomolecular interactions such as ELISA provide only end-point data not taking into consideration association and dissociation kinetics. Furthermore, they are often cumbersome and may require rigorous sample preparation.

Octet® BLI systems provide researchers the capability to detect interactions of a diverse range of biomolecules, from small molecules to proteins to viruses. The fluidics-free approach with a wide variety of off-the-shelf dip and read biosensors for rapid kinetic binding and concentration analysis enables the analysis of not only purified biomolecules, but even those in complex media such as cell culture supernatants and lysates. In addition, selected Octet® BLI systems are available with 21 CFR Part 11 software and a comprehensive GxP package for reliably operating in regulated environments in downstream bioprocessing and manufacturing processes.

**Additional Information (click to expand)**

- Features and Benefits
- Advantages
- Featured Assays


- Real-time binding kinetic and affinity measurements
- Measure interactions from purified and crude samples alike
- Fast data acquisiton — data from up to 96 biosensors, simultaneously
- Binding characteristics, concentration analysis to functional analysis, all in one platform

# Octet® Bio-Layer Interferometry

## Features and Benefits

| Feature | Benefit |
|---|---|
| High-quality kinetic screening and affinity charcterization | Accurate results |
| Microfluidics-free Dip and Read format reduces assay time and maintenance cost | Crude sample compatibility and low instrument maintenance |
| Up to 96 parallel, independent, channles for maximum speed and flexibility | Saves time and cost. The scalable throughput can be further increased by platform integration |
| Sensitivity to detect analytes from small molecules to viruses | Wide-ranging applications space |
| Non-dectructive sampling allows full sample recovery | Sample re-usability, saving reagent cost |
| Perfectly suied to operate in GxP-regulated environments | Faster to market |

# Octet® Bio-Layer Interferometry

## Advantages

- **High- Throughput** - Up to 96 samples can be processed simultaneously on the Octet® RH96 system
- **Speed** - Protein quantitation can be complete in as little as two minutes
- **Sample compatibility** - Analytes can be measured in unpurified mixtures such as cell lysates or hybridoma supernatants.
- **Lower Total Cost of Ownership** - Fluidic-free design prevents cleaning, high amount of maintenance, risk of cloggings which cause downtime while waiting for replacements

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**Additional Information (click to expand)**

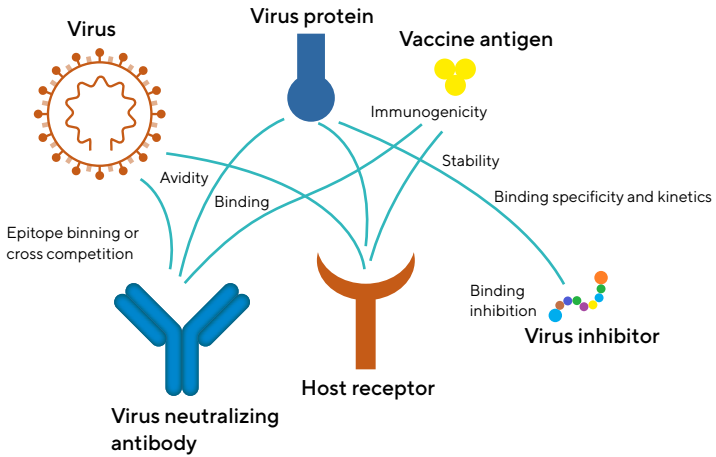
- Features and Benefits +
- Advantages +
- Featured Assays ×
  - Select best vaccine and therapeutic candidates early in the development process based on binding affinity and kinetics assessments
  - Octet® Assays in Neutralization Antibodies Development workflows
  - Fast and accurate determination of vaccine titer and potency

# Octet® Bio-Layer Interferometry

## Featured Assays

**1. Select best vaccine and therapeutic candidates early in the development process based on binding affinity and kinetics assessments**

Octet® BLI binding analysis supports a wide range of assays and workflows in vaccine development, virology and therapeutics development providing binding affinity, kinetics, specificity, stability indications, potency, binding epitope profiles and concentration analysis. The microfluidics-free design, and dip and read biosensors, provides unmatched ease-of-use and assay flexibility, speeding up data collection and results.

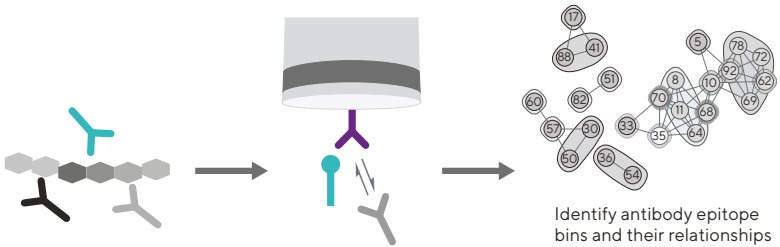


# Octet® Bio-Layer Interferometry

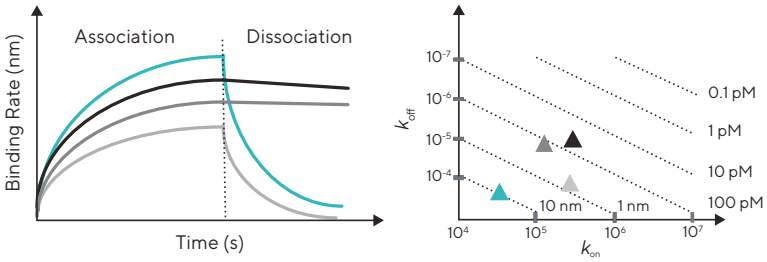
## Featured Assays

**2. Octet® BLI assays in neutralization antibodies development workflows**

Epitope binning assays help identify antibodies that block the same epitope on a target antigen and are crucial when it comes to identifying or engineering mAbs with favorable kinetics and affinity profiles. This assay format can also be used to determine neutralization antibody competition with virus receptor binding to host receptors.




Real-time Kinetics (association and dissociation) and affinity data allows to confidently pick target binding lead candidates fast. Binding activity measurements can also be used to determine protein stability and activity, especially useful in vaccine antigen selection.

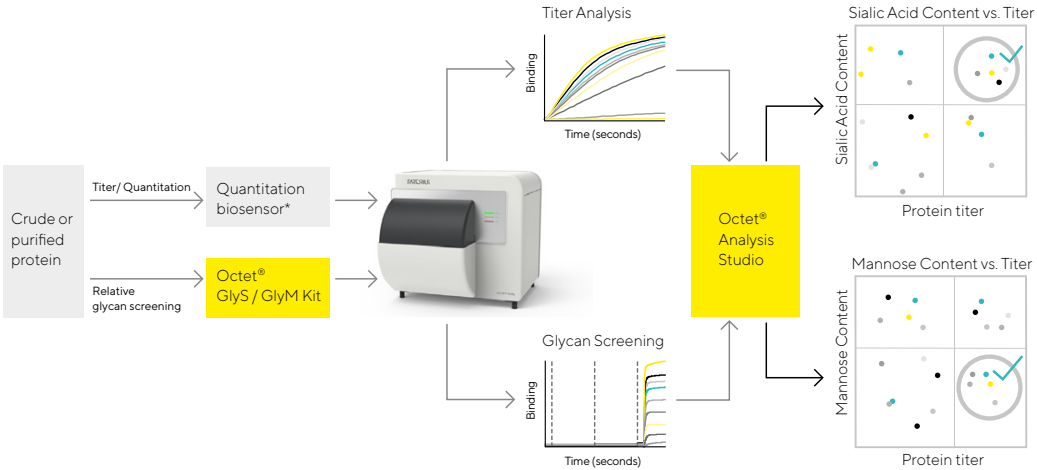



**Click for more**

# Octet® Bio-Layer Interferometry

## Featured Assays

**2. Octet® BLI assays in neutralization antibodies development workflow**



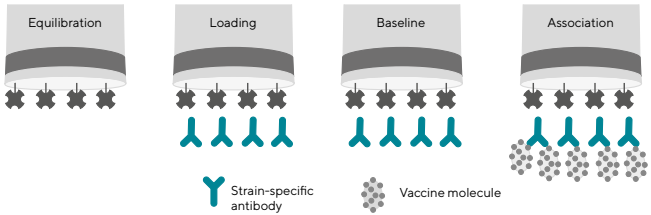

Octet® BLI biosensor assays are used for clone screening, titer analysis and relative screening for determining other critical quality attributes (CQAs) such as potency or relative screening of the glycosylation profile of biologicsdrug molecules. Crude sample compatibility of Octet® BLI assays enable early analysis of clone specificities, productivity and attributes for early decision making.

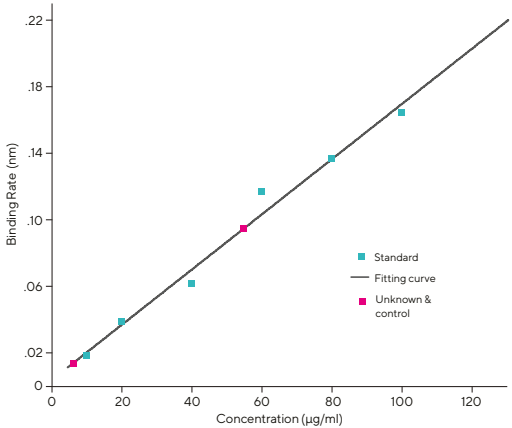
**Click to go back**

# Octet® Bio-Layer Interferometry

## Featured Assays

**3. Fast and accurate determination of vaccine titer and potency**




Fast and accurate determination of vaccine titer during manufacturing is important in understanding vaccine development process performance and for correctly scaling each process step. Vaccine titer provides a critical quality attribute to monitor the manufacturing process too. An Octet® BLI assay configuration to assess influenza vaccine potency is shown (left). Quantitation of vaccine potency of process lots is performed based on vaccine binding levels to a strain specific antibody is comparing it to a standard vaccine lot (right).

# Research & Testing Toolkit

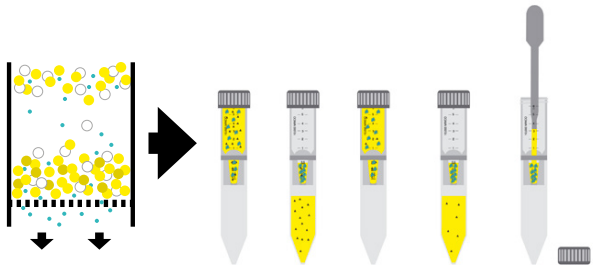
**From Mammalian Cell Cultures to Pure Proteins and Viruses**
Moving forward in the vaccine research and development workflow, the downstream process, such as clarification, sterile filtration and concentration of a target protein or virus can also bear various challenges. These processes can be very tedious and time-consuming.

**Additional Information (click to expand)**

- Protein and Virus Harvesting in One Step +
- Concentration and Purification of Viruses +
- Concentration, Purification, and Diafiltration of Proteins (Antigens and Antibodies) +

**Detection of Pathogens, such as SARS-CoV-2 in the Air**

- Air Sampling Tools +



# Research & Testing Toolkit

## Protein and Virus Harvesting in One Step with Sartoclear Dynamics® Lab V Kits

**Sartoclear Dynamics® Lab V Kits** combine single-use vacuum filtration units, Sartolab® RF with either a 0.2 µm or a 0.45 µm PES membrane and a diatomaceous earth (DE) filter aid for clarification of sample volumes of 50 mL up to 1 L, enabling clarification and sterile filtration to be performed in one step.

Learn More About Sartoclear Dynamics® Lab

The **Sartolab® Multistation** addresses critical pain points in handling of multiple samples specially designed to hold 1 to 6 vacuum filtration units, allowing simultaneous filtration of up to 6 samples.

- A single-step filtration with high flow rates
- Efficient: only one filter per filtration needed
- Versatile with solutions for all cell densities in 50 mL to 1 L volumes

eBook: Overcoming Challenges in High Density Cell Culture Harvesting During Antibody Development

# Research & Testing Toolkit

## Concentration and Purification of Viruses

During the preparation, handling, or analysis of viruses or virus-like particles (VLPs), a concentration and/or purification step is frequently required. Most viruses have a diameter within the range of about 20 nm up to several hundred nanometers, therefore they are ideally suited for retention using ultrafiltration membrane systems.

**Vivapure® Virus Purification and Concentration Kits**
Vivapure® Adenopack and Lentiselect purification kits utilize membrane adsorber technology for fast and safe virus purification. They are ideally suited and routinely used in vaccine research applications for the purification and concentration of adenovirus and in the volume range from 20 to 1,000 mL. The ready to use kits contain all buffers and devices needed for quantitative yield virus purification in only a few hours.

**Application Note: Ultrafiltration Methods for the Concentration and Purification of Viruses**

# Research & Testing Toolkit

## Concentration, Purification, and Diafiltration of Proteins (Antigens and Antibodies)

Vivaflow® and Vivaspin® ultrafiltration devices offer perfect solutions for any ultrafiltration needs in protein, peptide, nucleic acid, nanoparticle and virus purification workflows. From concentration and diafiltration of the original target molecule source, such as a cell culture supernatant, to final concentration, desalting or buffer exchange of the purified protein.

**Vivaspin® and Vivaflow® Ultrafiltration Devices**

- Concentrate initial sample volumes of 100 µL to 5 L
- Broad range of membrane types and molecular weight cut-offs (MWCO)
- Integral dead-stop prevents risk of sample loss from running to dryness


**Application Note: Ultrafiltration Methods for the Concentration and Purification of Viruses**

# Research & Testing Toolkit

## Detection of Pathogens such as SARS-CoV-2 in the Air

Sampling and detection of pathogens in the environment is not only a reliable component of a surveillance program and early warning system but also the first step of identifying the pathogen and its genetic material for vaccine development.

With the MD8 air samplers, the air of all contamination risk areas can be sampled to detect the presence of SARS-CoV-2. In combination with the water soluble gelatine membrane filters the MD8 air sampler enables reliable sampling followed by easy storage and sample preparation of viruses and other pathogens.

The unique water-soluble gelatine membrane filters are the perfect way to easily capture SARS-CoV-2 and other airborne pathogens in the surrounding air and provides the highest retention rates for bacteria, viruses, spores, and phages and maintains the viability of the sampled microorganisms and viruses.

**Easy Steps from Collection to PCR Detection of Virus**

**Preparation for sampling**

**Active on-site air sampling**

**Membrane transfer**

**Membrane dissolving**

**Extraction and PCR amplification**

See how experts have used Sartorius air samplers and Gelatine Membrane Filters in different virus detection areas

# Summary

The recent outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus, SARS-CoV-2, and its rapid spread across the globe has spurred an increased focus in developing new vaccines and therapeutic drugs against viruses. Currently, various innovative approaches are being used to target different antigenic epitopes of the SARS-CoV-2 proteome, to develop vaccines which are effective against the growing number of variants.

Such developments begin by establishing the intricate details of host-virus interaction at the cellular and molecular level, to gain a deep understanding of the mechanisms that drive immune responses against the virus and its variants.

In this eBook, we outline some key applications in virology and vaccine research, including elucidation of interactions between the SARS-CoV-2 spike protein and its receptor on host cells, and our current understanding of humoral and T-cell immune responses following vaccination.

Sartorius continues to support and simplify these early stage virology and vaccine research and development efforts. From the innovative Incucyte® live-cell analysis systems, to iQue® advanced flow cytometry, and Octet® label-free biomolecular interactions analysis platforms, vaccine developers can gain critical new information, with confidence, as quickly as possible.

In addition, vaccine development requires platforms that can overcome various challenges in downstream processes. For this purpose, Sartorius offers solutions for clarification and sterile filtration of low to intermediate cell culture volumes, and a broad portfolio of ultrafiltration devices serving the complete protein ultrafiltration workflow - from concentration and diafiltration of the original protein source, to final concentration/ desalting of the purified protein.

**References**

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3. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2 | Science (sciencemag.org)
4. Potently neutralizing human antibodies that block SARS-CoV-2 receptor binding and protect animals | bioRxiv

**Germany**
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

**USA**
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com/air-monitoring


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