MOLECULAR DEVICES

The ultimate GUIDE to microplate reader solutions

Quick Overview

For over 37 years, we have partnered with scientists to expand the boundaries of their research. Our microplate readers and software are the industry's most cited and have empowered life science researchers to advance protein and cell biology–breaking the barriers to novel, landmark discoveries. This guide will help you on your journey towards turning your research into results.

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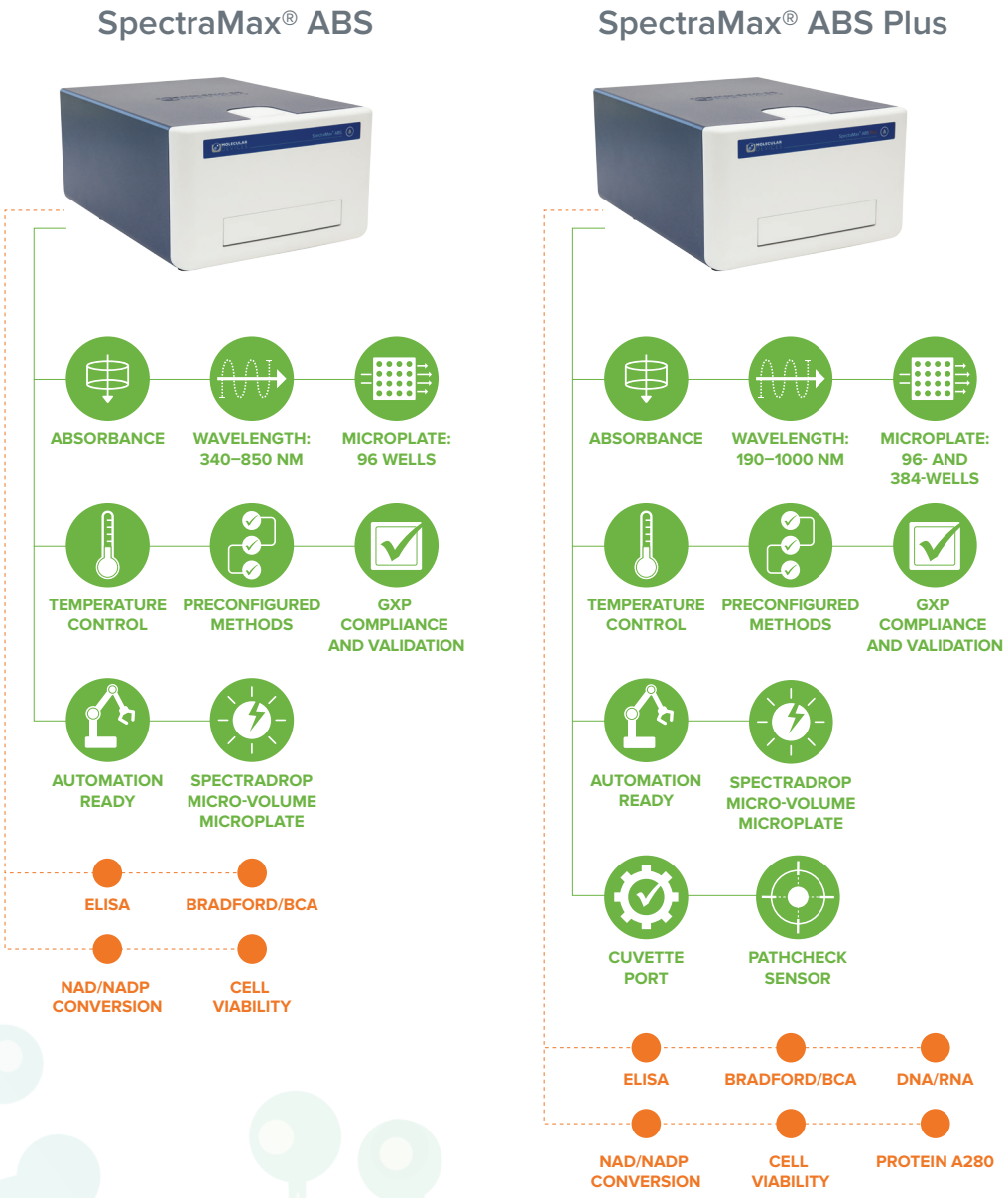
Introduction to microplate readers

Evaluating microplate readers doesn't have to be overwhelming. First, consider your application needs. If your budget is modest, a single-mode reader dedicated to your main application may be the most logical choice. If you are working with—or anticipate exploring—a greater variety of applications, a multi-mode reader would be the better choice.

What is a single-mode microplate reader?

The three most common read modes in a single-mode microplate reader are absorbance, fluorescence, and luminescence. A single-mode microplate reader is a good starting point. However, an upgradeable multi-mode reader can address all your future lab needs. You may ask yourself, what's the difference between an absorbance spectrophotometer and a microplate reader? A standard spectrophotometer measures the absorbance of one sample at a time. The sample is typically placed in a cuvette through which light is sent horizontally. An absorbance plate reader offers higher throughput and can measure the absorbance of samples in microplates (typically 96-well or even 384-well) by sending light through each well vertically. Similarly, a luminescence or fluorescence microplate reader increases your throughput manyfold over a single tube detection system.

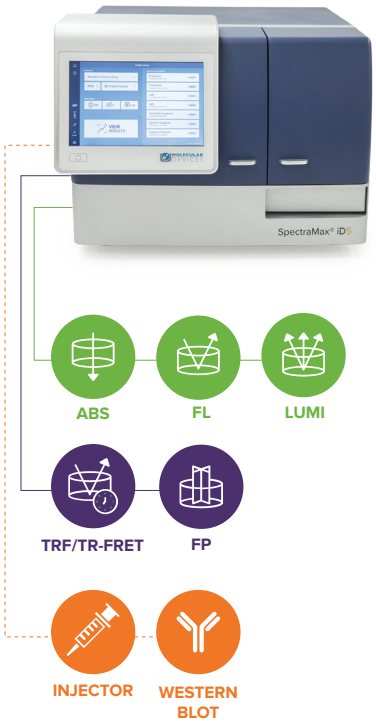


What is a multi-mode microplate reader?

A microplate reader with two or more detection modes, e.g. absorbance and fluorescence, is considered a multi-mode reader. Multi-mode readers have many advantages for labs conducting applications ranging from ELISAs to nucleic acid and protein quantitation to cell imaging. Combining multiple microplate technologies and detection modes into a single, more versatile unit may be an ideal choice, especially if you have limited lab space. A multi-mode reader can detect absorbance, luminescence, fluorescence, and more specialized measurements such as time-resolved fluorescence (TRF), fluorescence polarization (FP), Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), and Homogeneous Time-Resolved Fluorescence (HTRF). Other technologies such as imaging, Alphascreen®/AlphaLisa®, or western blot detection can be added to some multi-mode plate readers, increasing their flexibility. Consider a system that has the ability to be upgraded if your current budget is a factor.

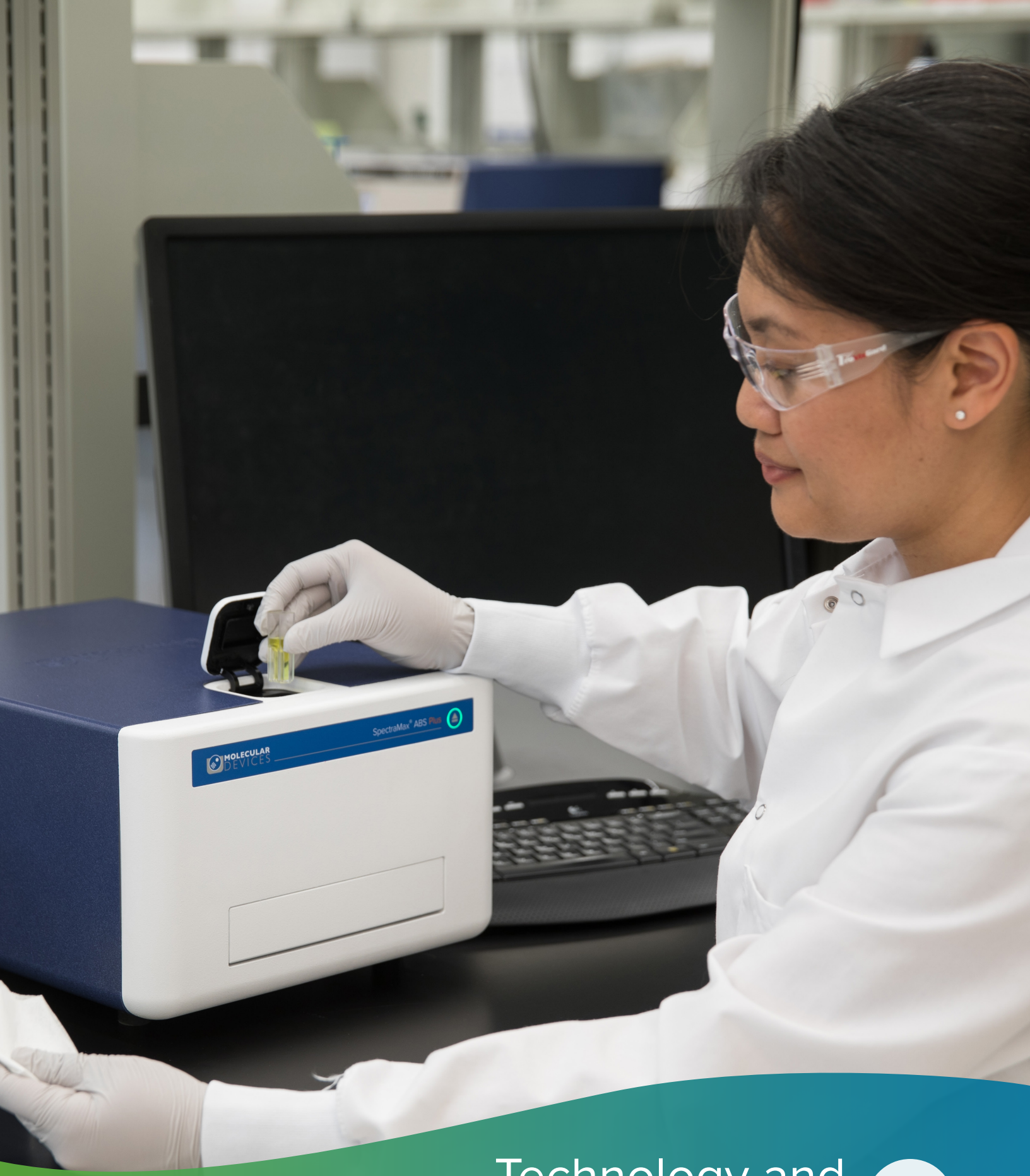
SpectraMax® i3x

SpectraMax® iD5



SpectraMax® iD3

SpectraMax® M5



Technology and detection modes

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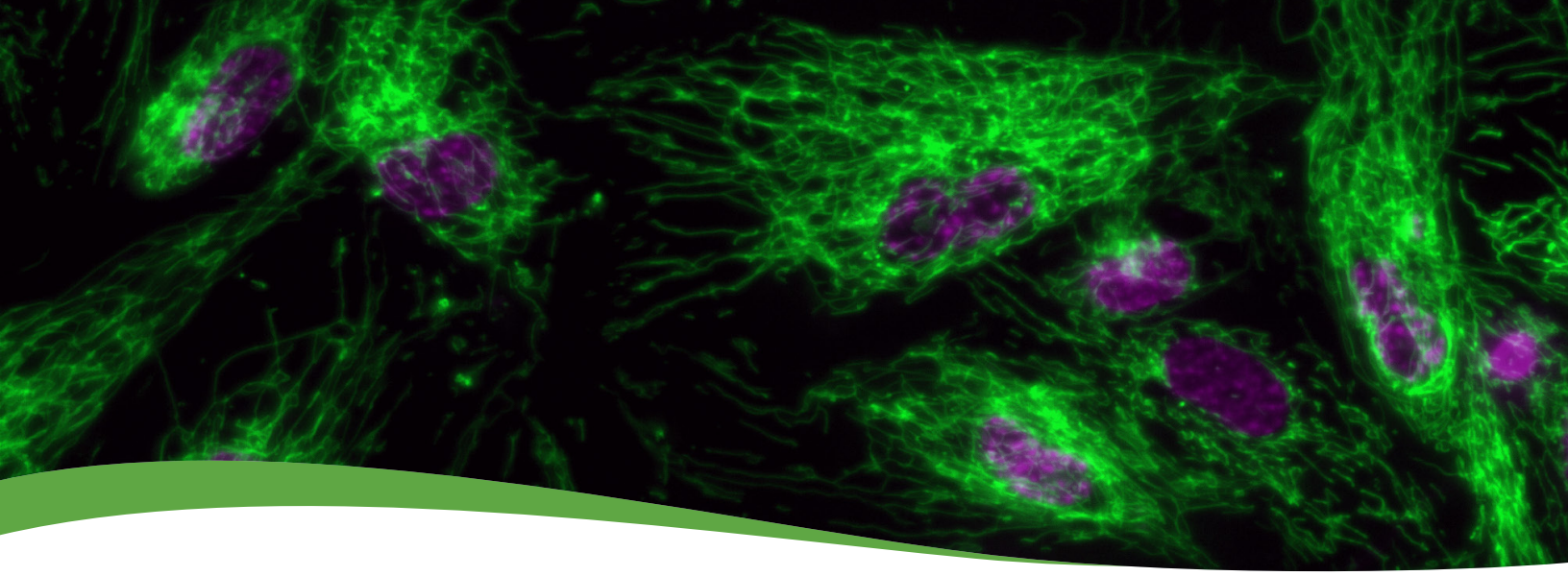


Technology and detection modes

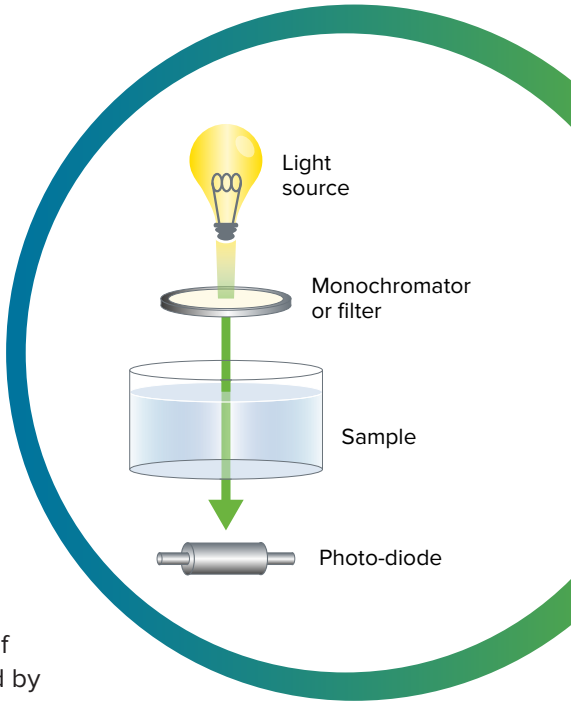
When choosing a microplate reader, one of the most important things to consider is your application requirements. Microplate readers are widely used in research, drug discovery, bioassay validation, quality control, and manufacturing processes in the pharmaceutical, biotechnology, food and beverage, and academic environments. They enable the measurement of a variety of analytes for a wide range of assays including quantitation, binding, cell signaling, and more. Continue reading below to discover the core technology and detection modes for microplate readers and how they work.

a. Absorbance

What is absorbance and how does it work?

Absorbance, also known as optical density (OD), is the quantity of light or a particular wavelength that is absorbed by a solution. Changes in absorbance due to enzymatic reactions, or the presence of specific analytes in a sample, can be detected using a microplate reader.

Ultraviolet (UV) measurements in microplates became possible when Molecular Devices introduced the first UV-capable microplate reader. Since then, direct measurements of DNA, RNA, and proteins in microplates have become very popular.



How does absorbance detection work?

Spectrophotometers and absorbance microplate readers measure how much light is absorbed by a sample at a particular wavelength. Microplate readers that are capable of detecting light in the UV range can be used to determine the concentration of nucleic acids (DNA and RNA) or protein directly, without the need for sample labeling. Light of a certain wavelength, dependent on the analyte being measured, is passed through a sample, and a detector on the other side of the microplate well measures how much of the original light was absorbed by the sample in the well.

Learn more about **absorbance detection** and how it works

b. Fluorescence

What is fluorescence?

Fluorescence is the property of some substances to absorb light at a particular wavelength (the excitation: (Ex), followed by emission (Em) of light at a longer wavelength (Figure 2). The distance between the excitation and emission peaks is known as the Stokes shift and is fluorophore-dependent (Figure 1).

Fluorescence involves exciting a sample at a particular wavelength. When excited at the appropriate wavelength, the molecule is transformed from a ground to an excited state. As the molecule returns to the ground state, energy is released in the form of heat and light at a different longer wavelength of lower energy (Figure 3).

How does fluorescence detection work?

A microplate reader with fluorescence intensity (FI) detection uses a light source, usually a xenon flash lamp or LED, to excite a fluorophore (fluorescent molecule) at a particular wavelength. The wavelength required to excite the sample can be selected using either a filter for a specific wavelength or a monochromator tuned to the required wavelength.

The fluorophore then emits light of a different (higher) wavelength, which is selected by a second filter or monochromator. This emitted fluorescence is detected by a photomultiplier tube (PMT), and the fluorescence intensity of the sample is expressed as relative fluorescence units.

Learn more about **fluorescence detection** and how it works

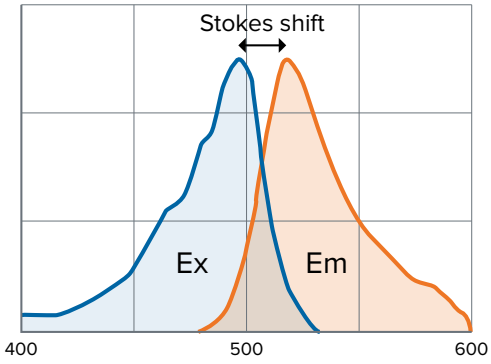


Figure 1

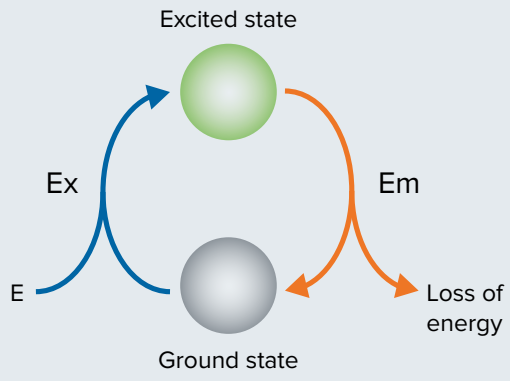


Figure 2

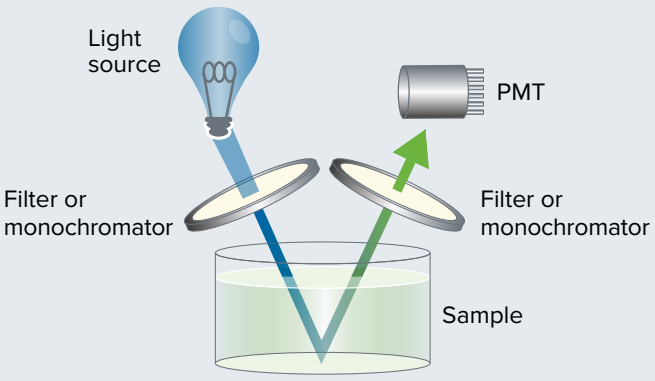
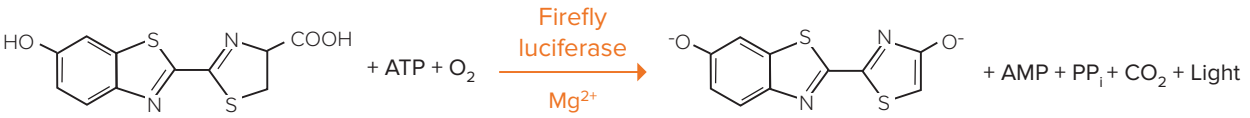


Figure 3

c. Luminescence

What is luminescence?

Luminescence is the emission of light by a substance as a result of a chemical reaction (chemiluminescence) or an enzymatic reaction (bioluminescence).



Luciferin Oxyluciferin

Luminescence detection is optically simpler than fluorescence detection as it does not require a light source or specific optics for excitation.

Luminescence can be either a 'flash' or a 'glow' reaction, depending on the kinetic profiles. Flash luminescence gives a very bright signal for a short amount of time, usually seconds. Glow luminescence emits a more stable but usually less intense signal that can last for several minutes or hours (Figure 1). Flash luminescence generally requires a detection system with injectors that can deliver substrate to the reaction shortly before taking a measurement so that signal is not missed. White opaque microplates are usually recommended for luminescence, as they reflect light and maximize the amount of signal detected.

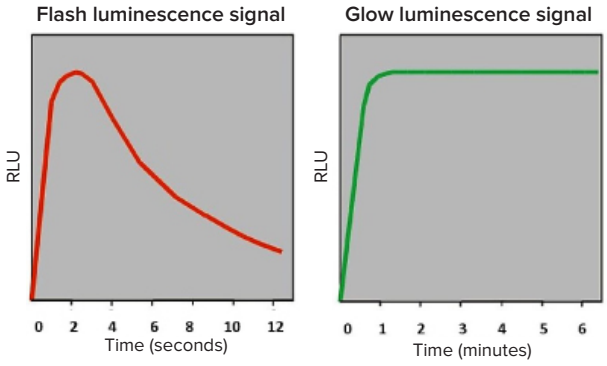


Figure 1. *Flash and glow reactions.*

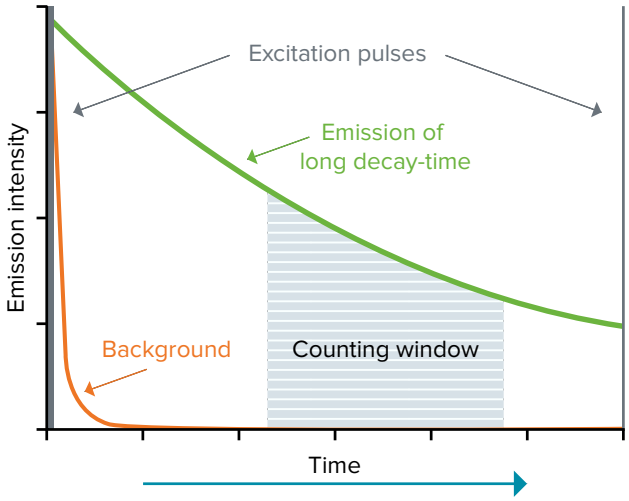
Learn more about **luminescence detection** and how it works

d. TRF/TR-FRET

Time-Resolved Fluorescence (TRF)

Fluorescence intensity (FI) measurements use standard fluorophores like fluorescein, whose emission is short-lived, on the order of nanoseconds. Excitation of the sample and measurement of emission occur simultaneously. Although microplate readers are very good at screening out excitation light from the emission measurement, short-lived light emitted by materials in the well or sample often contributes to high background.

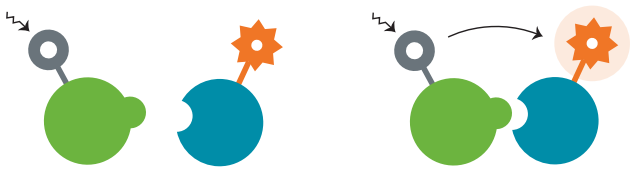
Time-resolved fluorescence (TRF) reduces background by using a lanthanide fluorophore, such as europium, terbium or samarium, which emits long-lived fluorescence. This long-lived fluorescence lasts for milliseconds, so excitation of the fluorophore by a pulsed light source (e.g., a flash lamp), followed by a delay and then signal measurement (counting window), allows short-lived fluorescence (lasting only for nanoseconds) to subside before a measurement is made. Assays using time-resolved fluorescence offer dramatically reduced signal-to-noise ratios. Lanthanide fluorophores are commonly used as chelate or cryptate complexes that enable good signal intensity and stability.



Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

TR-FRET combines the time-resolved (TR) measurement of fluorescence with fluorescence, or Förster, resonance energy transfer (FRET) technology. In FRET assays, biomolecules (e.g., proteins) are labeled with donor and acceptor fluorophores. When the biomolecules interact, donor and acceptor fluorophores are brought close together. Now, when the donor is excited, it can transfer energy to the acceptor, which in turn emits fluorescence at a specific wavelength. Acceptor and donor fluorescence emissions have different wavelengths that can be distinguished from each other by a microplate reader, and the ratio of acceptor to donor emission is a quantifiable indicator of biomolecular interaction.

Using lanthanide fluorophores, which have long-lived fluorescence emission, as donors, TR-FRET assays take advantage of the time-resolved measurement of fluorescence to eliminate short-lived background fluorescence. In a TR-FRET assay, thanks to the donor fluorophore's long-lived emission, excitation and emission of both donor and acceptor fluorophores can also be measured after short-lived background fluorescence has abated.

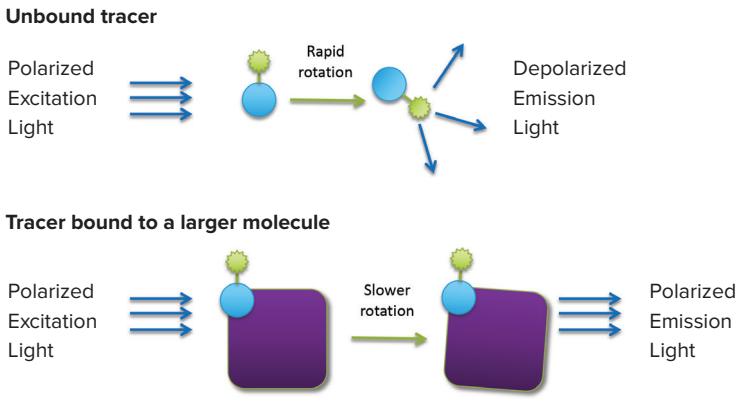


Learn more about **TRF/TR-FRET** and how it works

e. Fluorescence Polarization (FP)

Fluorescence polarization (FP) is a detection mode that is widely used to monitor binding events in solution. It can be used to assess biomolecular interactions, including protein-antibody binding and DNA hybridization, as well as enzyme activity, and it has been adapted to basic research as well as high-throughput screening.

A small, fluorescently labeled molecule (tracer) that is excited with plane-polarized light emits mostly depolarized light because the tracer tumbles rapidly during the time between excitation and emission. However, when the tracer binds a much larger molecule, it rotates more slowly, and the emitted light remains largely polarized. Measurement of the light emitted in parallel and perpendicular directions to the excitation light is used to determine the amount of binding.



Learn more about **fluorescence polarization** and how it works

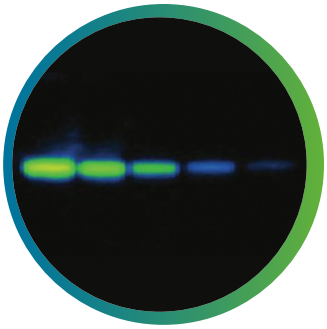
f. Western blot

What is western blotting?

Western blotting is a popular technique used for protein detection and quantitation. It enables the separation and identification of a specific protein of interest from a complex mixture of proteins, for example a cell lysate. With applications in diagnostics, biotechnology, molecular biology, proteomics, and much more, western blots are widely relied upon to evaluate levels of protein expression in cells, as well as changes in size and other properties.

Western blotting typically employs two antibodies, a primary antibody that binds to the protein of interest and a secondary antibody that is conjugated to a detectable molecule. Depending upon the secondary antibody used, detection of the target protein may be colorimetric, chemiluminescent, or fluorescent. These methods require different instrumentation for detection. For example, chemiluminescence can be detected using x-ray film or digital imaging equipment, while a fluorescent secondary antibody requires a fluorescence imager. Each type of detection has advantages and disadvantages that need to be considered when selecting a method. Western blot detection on a multi-mode microplate reader is made possible with the ScanLater™ Western Blot Detection System.

The ScanLater system is a time-resolved fluorescence (TRF)-based western blot detection assay, an optimal detection method that offers higher sensitivity, and exceptional stability. The ScanLater system comprises the ScanLater Western Blot Detection Cartridge, ScanLater Western Blot Kit, and image acquisition software powered by SoftMax® Pro Software.



Learn more about **western blots** using your plate reader with ScanLater Western Blot Detection System

g. Injectors

Expand your lab's capabilities to include flash applications such as dual luciferase and ATP assays with on-board injectors. Our injectors are completely integrated, and are engineered for high performance with SmartInject® Technology and overflow protection. SmartInject technology ensures thorough reagent mixing for more consistent results, and overflow protection prevents damage caused by reagent spillage.

h. Cuvette ports

A cuvette port offers easy single-sample measurements. A microplate reader equipped with a cuvette port can be a great way to transition from low-throughput, single-tube assays to higher-throughput microplate-based assays. Our built-in cuvette port adds convenience and flexibility. The SpectraMax M Series supports cuvette reading in absorbance, fluorescence, and luminescence read modes with temperature control.

i. Imaging cytometry

An imaging cytometer enables image-based analysis of cells and gives you a more detailed view of phenotypic changes that accompany cytotoxicity, cell proliferation, and protein expression. StainFree™ Cell Detection Technology, additional brightfield analysis features, and fluorescent green and red detection channels are highlights of the SpectraMax MiniMax™ 300 Imaging Cytometer.

The SpectraMax MiniMax cytometer option is a field-upgradeable option for the SpectraMax i3/i3x Multi-Mode Microplate Reader that offers cellular imaging capability.

Simplified workflow

Add cellular imaging assays in a small footprint

Stain-free analysis

Eliminate cell staining for cell counting and confluence measurements using StainFree Technology

More data

Multiparametric cell-based assays with brightfield and green and red fluorescent imaging

Familiar software

Set up, image, and analyze cells in a snap using SoftMax Pro Software

Improved data

Normalize your plate reader assay data to the number of cells in each well

GxP software compatible

Provides tools to achieve FDA 21 CFR Part 11 compliance for data generated by SpectraMax MiniMax cytometer

Consider your application needs

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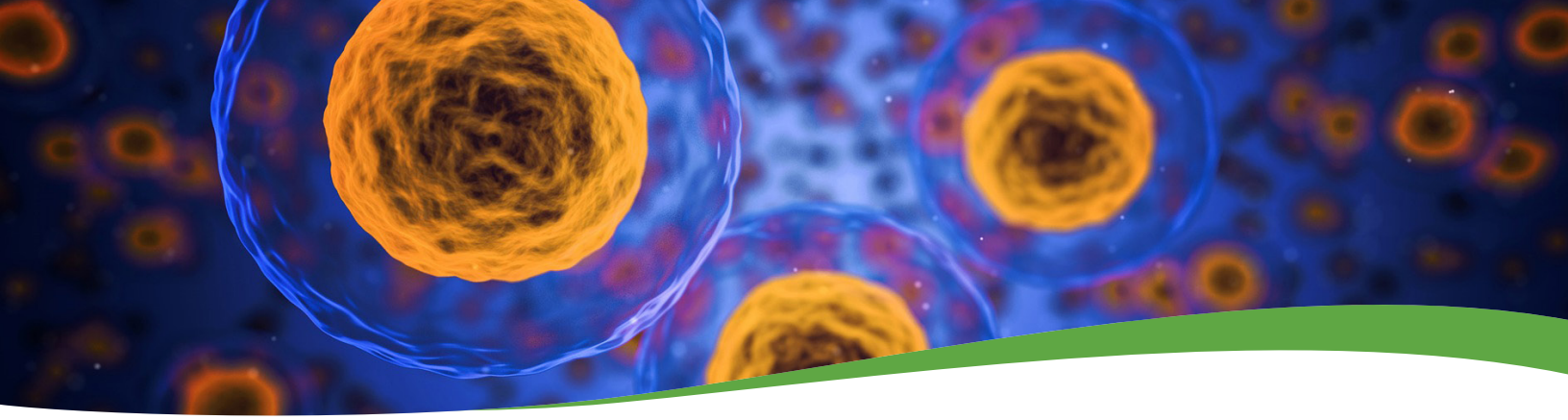


Consider your application needs

a. Cell viability

What is cell viability?

Cell viability refers to the number of healthy cells in a population and can be evaluated using assays that measure enzyme activity, cell membrane integrity, ATP production, and other indicators. These methods can employ luminescent, fluorescent, or colorimetric readouts as indicators of general cell viability or even specific cellular pathways. Cytotoxicity and cell viability assays are often used to assess a drug or other treatment's effect, and are valuable tools in the search for new therapeutics, as well as advancing our understanding of how normal cells function.

Learn more about **cell viability**, cell proliferation, and cytotoxicity assays

b. Cell signaling

What is cellular signaling and cellular response?

Cellular signaling allows cells to respond to their environment as well as to communicate with other cells. Proteins located on the cell surface can receive signals from the surroundings and transmit information into the cell via a series of protein interactions and biochemical reactions that comprise a signaling pathway. Multicellular organisms rely upon an extensive array of signaling pathways to coordinate the proper growth, regulation, and functioning of cells and tissues. If signaling between or within cells is dysregulated, inappropriate cellular responses may lead to cancer and other diseases.

Learn more about **cell signaling**

c. Cell migration

What is cell migration?

The movement or migration of cells is often measured *in vitro* to elucidate the mechanisms of various physiological activities such as wound healing or cancer cell metastasis. Cell migration assays may be conducted in a controlled environment using live cell, time-lapse imaging. A "wound" in a confluent monolayer of cells growing in a microplate is created, either by manually creating a scratch or by utilizing special microplates that provide a uniform and reproducible cell-free zone. Monitor cell proliferation, migration, and spreading using transmitted light or live cell-compatible fluorescence. These medium- to high-throughput assays may be used to study the migration of cells treated with either inhibitory or stimulatory compounds.

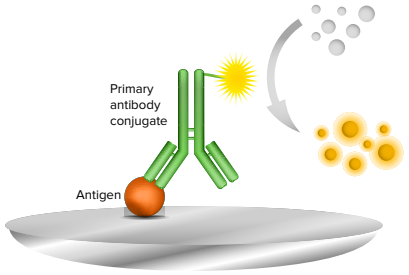
Learn more about **cell migration**

d. Enzyme-Linked Immunosorbent Assay (ELISA)

What is ELISA?

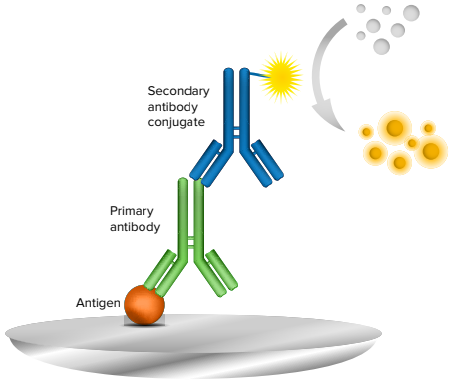
ELISA (enzyme-linked immunosorbent assay) is a method used to quantitatively detect an antigen within a sample. An antigen is a toxin or other foreign substance, for example a flu virus or environmental contaminant, that causes the vertebrate immune system to mount a defensive response. The range of potential antigens is vast, so ELISAs are used in many areas of research and testing to detect and quantify antigens in a wide variety of sample types. Cell lysates, blood samples, food items, and more can be analyzed for specific substances of interest using ELISAs.

There are four major types of ELISAs: direct, indirect, competitive, and sandwich. Each type is described below with a diagram illustrating how they work.



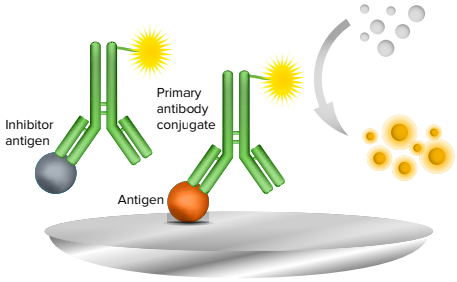
Direct ELISA

In a direct ELISA, the antigen is bound to the bottom of the microplate well, and then it is bound by an antibody that is specific to the antigen and also conjugated to an enzyme or other molecule that enables detection.



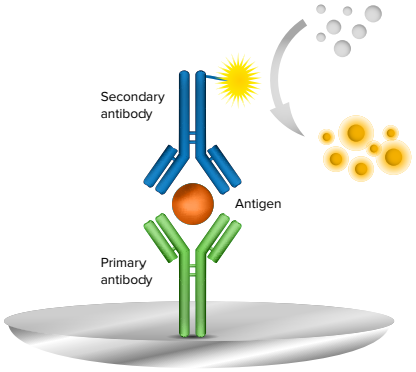
Indirect ELISA

In an indirect ELISA, the antigen is bound to the bottom of the microplate well, then an antibody specific to the antigen is added. A secondary antibody, conjugated to an enzyme or other detection molecule, is then bound to the first antibody.



Competitive ELISA

In a competitive ELISA, a reference antigen is bound to the bottom of microplate wells. Sample plus antibody are added to the wells, and if there is antigen present in the sample, it competes with reference antigen for binding to the antibody. Unbound material is washed away. The more antigen is in the sample, the less antibody ends up bound to the bottom of the wells by the reference antigen, and the lower the signal.



Sandwich ELISA

For the sandwich ELISA, two antibodies specific to two different epitopes on the target antigen are used. The capture antibody is bound to the bottom of the microplate well and binds one epitope of the antigen. The detection antibody binds to the antigen at a different epitope and is conjugated to an enzyme that enables detection. (If the detection antibody is unconjugated, then a secondary enzyme-conjugated detection antibody is required).

Learn more about **ELISAs**

e. Microbiology and contaminant monitoring

Monitoring microbial growth and contaminants

Microbes, including bacteria, have been estimated to make up about 15 percent of the earth's biomass, and microbes in the human body outnumber human cells by 10 to 1. These microorganisms provide great benefit to us and are also vital to many fields of research from medicine to alternative energy production. On the other hand, monitoring for microbes and the toxic substances they produce is necessary to ensure the safety of pharmaceutical products. Scientists whose research relies on mammalian cells must carefully monitor these cultures for unwanted microbial contaminants to ensure that their experimental results are reliable.

Learn more about **microbiology and contaminant monitoring**

f. Nucleic acid (DNA/RNA) quantitation & analysis

What are nucleic acids?

Nucleic acids are large biomolecules common to all known life forms. Deoxyribonucleic acid (DNA) consists of a double strand of pairs of nucleotides, while ribonucleic acid (RNA) is typically a single strand. In DNA, the nucleotides are adenine, cytosine, guanine, and thymine, while RNA contains uracil instead of thymine. DNA makes up the genetic material of most organisms, encoding the information cells need to synthesize proteins.

Nucleic acid is often purified from cells as part of an ever-growing array of molecular biology methods. Before they are used in downstream applications, nucleic acids are quantitated using UV or fluorescence spectrophotometry. Traditionally measured individually in cuvettes, sample analysis is now routinely performed in microplates.

Learn more about **nucleic acid (DNA/RNA) quantitation & analysis**

g. Protein quantitation & analysis

Detect, quantify, and analyze proteins

Detection, quantitation, and analysis of proteins are central to the investigation of a wide variety of biological processes. Measuring the concentration of protein is necessary to processes ranging from protein purification and labeling to sample preparation for electrophoresis. Protein can be quantitated directly via absorbance at 280 nm, or indirectly using colorimetric (BCA, Bradford, etc.) or fluorometric methods offering advantages such as greater sensitivity.

Cellular signaling and other biological processes may be analyzed using fluorescent proteins. For example, green fluorescent protein (GFP) can be expressed in living cells and used to visualize protein localization and dynamics under experimental conditions. Tryptophan, an amino acid whose fluorescence emission properties are affected by its microenvironment, has also been used to monitor changes in the conformational state of proteins.

Learn more about **protein quantitation & analysis**

GxP compliance solutions for GMP/GLP labs

4

GxP compliance solutions for GMP/GLP labs

Laboratories operating under GMP (good manufacturing practice) and GLP (good laboratory practice) regulations must follow FDA or regional regulatory guidelines to protect data integrity or demonstrate quality assurance of manufactured products. To be GxP (good practice) compliant, regulated labs using microplate readers in their workflows need to verify that they are functioning within operational specifications and that the data acquisition and analysis software complies with regulations for electronic recordkeeping.

Molecular Devices is a leader in comprehensive compliance solutions with microplate detection systems and software. Combined with validation services and support, our solutions assure data integrity.

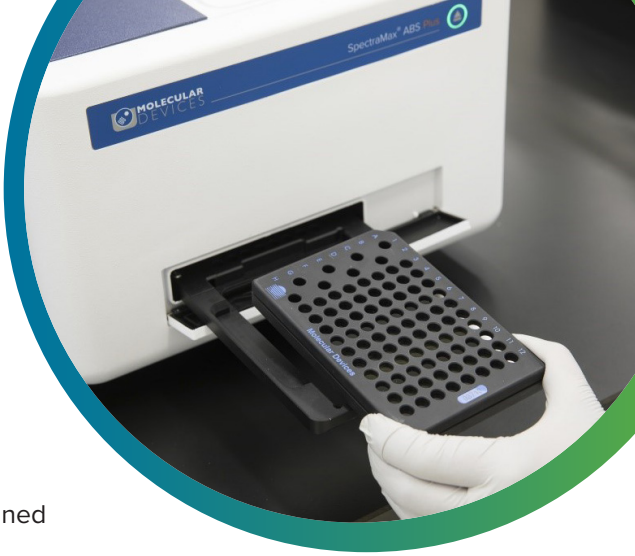
a. SoftMax Pro GxP Software

Become compliant with FDA part 11 software validation

SoftMax® Pro 7.1.2 GxP Software is the latest, most secure software to achieve full FDA 21 CFR Part 11 compliance with streamlined workflows to assure data integrity. Every step is optimized to simplify analysis and reporting to support our microplate readers.

Our expert team will partner with you to set up single- or enterprise-level software, and provide installation qualification (IQ) and operational qualification (OQ) services using our validation package to establish full compliance on your microplate readers. Major data privacy and security improvements support the latest General Data Protection Regulation regulations. See how **SoftMax Pro GxP Software** helps you meet **21 CFR Part 11 and EU GMP Annex/11** requirements.

Learn more about how our expert team can help you become and remain compliant to the latest **GxP standards**



b. Software installation and validation

Our software installation services verify and document that required components are installed to operational specifications. SoftMax Pro GxP Software can be installed on a single computer or on a multi-computer networked environment by our expert technical support or professional services team via remote access, respectively.

Our on-site SoftMax Pro GxP Software validation service supports FDA 21 CFR Part 11 guidelines and is conducted by our certified Field Service Engineer (FSE). Each step in the process will be carefully planned and executed.

Learn more about this and other **GxP services**

c. Validation plates & recertification services

SpectraTest Validation Plates

Molecular Devices microplate readers are designed to provide consistent performance for many years. In keeping with best practices, you must periodically validate and document the instrument performance to fulfill regulatory requirements. Our SpectraTest® Validation Plates are valuable tools for verifying that microplate readers are operating correctly in GMP and GLP laboratories. You can use photometric accuracy tests to verify how close an absorbance measurement is to the true value, which is traceable to the National Institute of Standards and Technology (NIST) and NMI (National Metrology Institutes). Additionally, you can determine the accuracy and repeatability of the wavelength selection with the monochromator, including separate tests for excitation and emission. Finally, you can qualify the kinetic noise that measures the stability of the optical system at low and high signals, including separate tests for spikes and drift at low and high levels.

Learn more by watching our **SpectraTest ABS2 Validation Plate** overview video

d. IQ/OQ/PM services

Installation qualification (IQ), operational qualification (OQ), preventive maintenance (PM), and repair coverage for microplate readers and washers.

IQ/OQ services and PM/OQ service plans preserve instrument documentation in a digital and compliant format. Assure ongoing compliance of your Molecular Devices microplate readers and be audit ready with comprehensive validation, maintenance, and repair documentation.

Learn more about **IQ/OQ/PM services**, and how they can help your lab

e. FDA 21 CFR Part 11 and the importance of regulatory compliance in GMP and GLP labs

The regulations for food and drug in the United States, described in the Title 21 of the Code of Federal Regulations, are critical in ensuring safe and ethical drug administration. Whether you are an academic institution, a government agency, or a pharmaceutical company, you have to adhere to these rules at each step of the drug development process. Failure to do so could even cascade into a corporate shutdown in the long run.

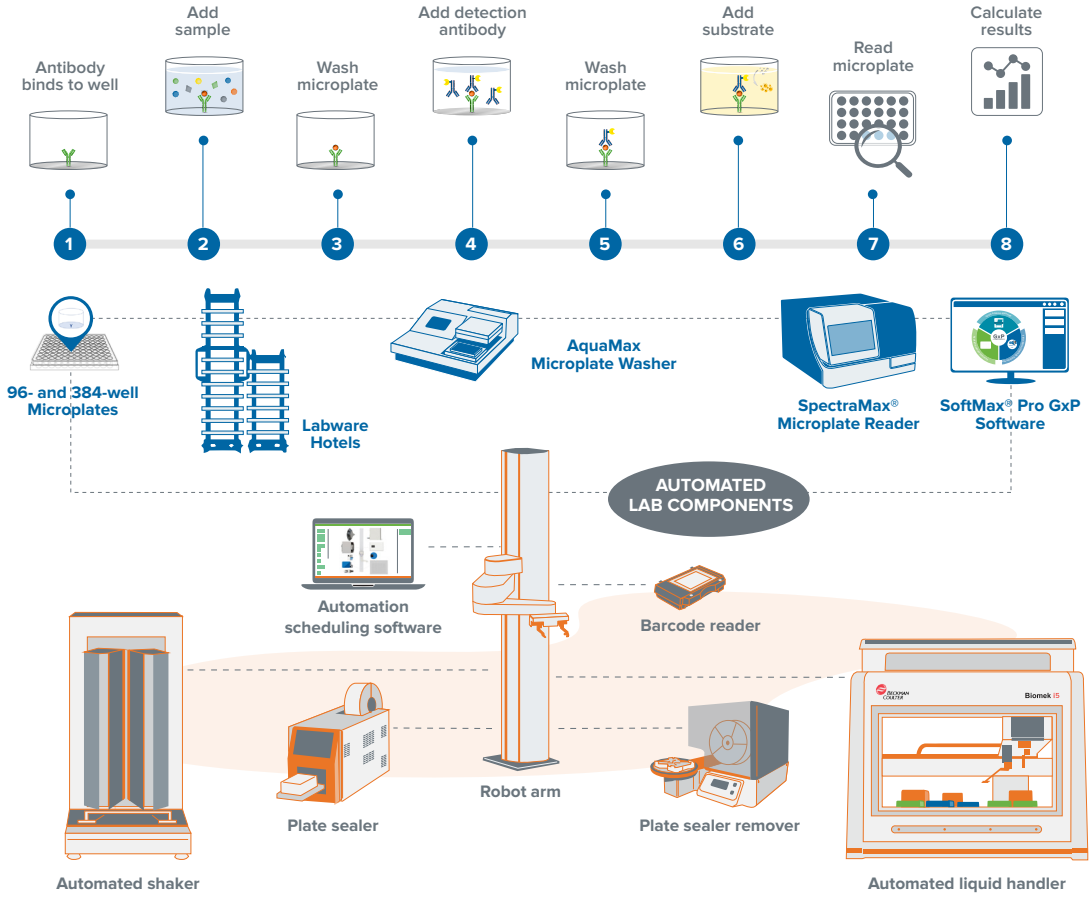
Read our **Lab Notes blog article** for a detailed breakdown of the essential components of Regulatory Compliance in GxP labs and our approach to achieving and maintaining these standards

f. Lab automation solutions for high-throughput workflows

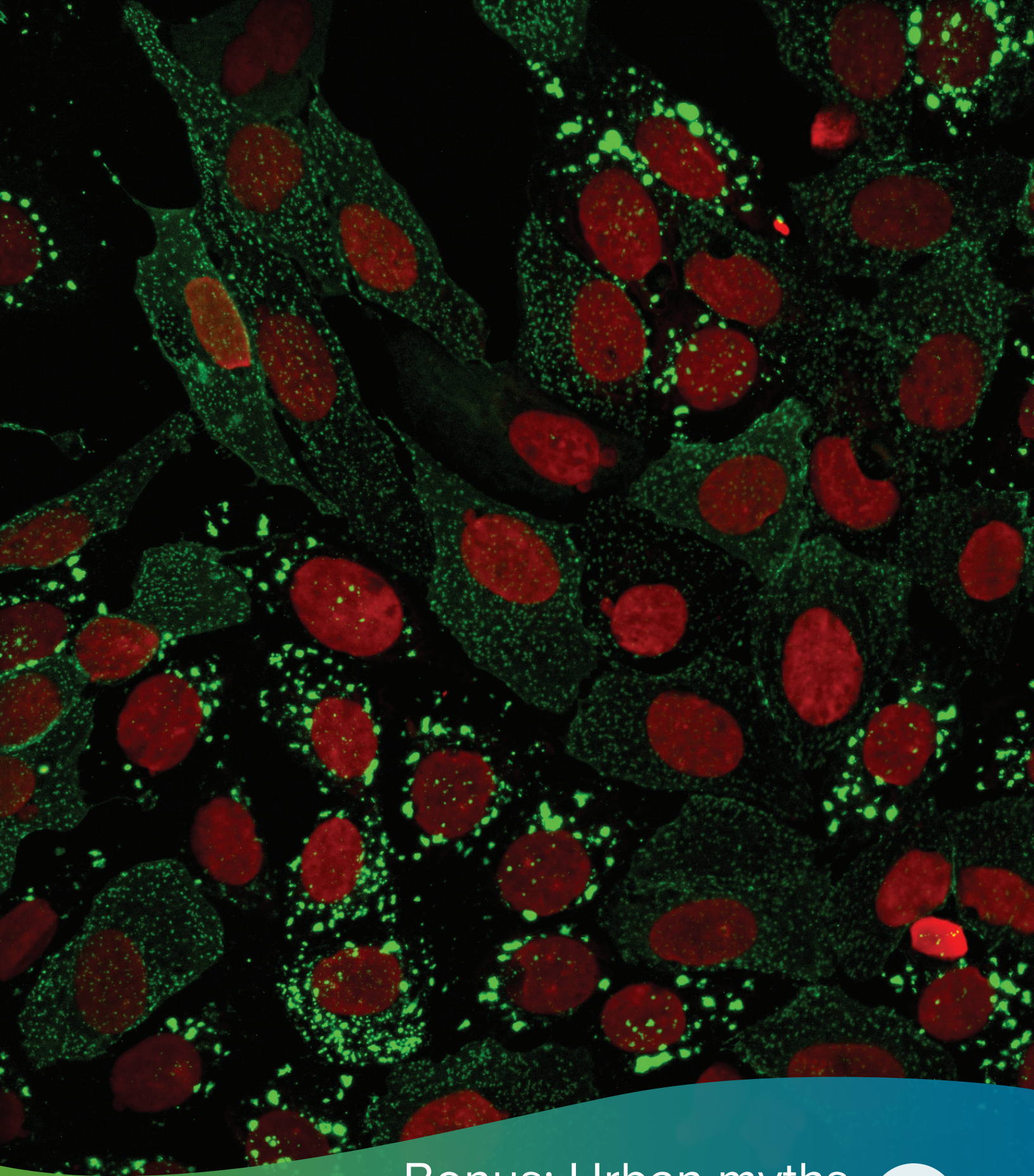
Our automation and customization solutions involve scientists and engineers who can customize our instruments, as well as automate entire workflows to meet the specific needs of your assay, method, or protocol. We take a consultative approach to understand your application requirements and recommend labware, lab robotics, and software solutions that best match the unique needs of your application.

ELISA Workflow

ELISA is one of the most popular quantitative methods to detect a target antigen such as toxin or foreign substance within a sample. The assay is easy to set up and the range of potential analytes is vast, but the assay procedure is time consuming and labor intensive.



Discover how **laboratory automation workflows** can help with providing walkaway time, increasing throughput, effectiveness and efficiency of the assay procedure, and reproducibility



Bonus: Urban myths of microplate readers

5

Bonus: Urban myths of microplate readers

a. OD, RFU or RLU

What exactly are they and why bigger is not always better!

In this first urban myths of microplate readers webinar, we'll introduce you to the presenters who hope to educate (and entertain) you throughout this five part series—starting with a brief discussion of the three main read modes : absorbance, fluorescence and luminescence.

b. Which microplate reader?

Decisions, decisions, and how to be less confused!

There is an enormous range of microplate readers and microplate technologies available nowadays, so how do you know which is best for you? In this webinar, we will review the various microplate reader options available for fluorescence and look at the impact they may have on your data.

c. "Optimization? But the manual says I need to excite at 490 nm!"

Everyone wants to get the best data they can from their assay, but sometimes it can be difficult to know how to do this—especially, for example, when a reagent supplier provides a perfect protocol with the 'exact' settings you should use. In this webinar, we discuss why sometimes you should ignore this! We will also delve into some of the software settings in SoftMax Pro Software that will enable you to generate textbook quality data!

d. Beyond the basics

Real time, resolving time and transferring energy

In this webinar, we move beyond absorbance, fluorescence, and luminescence to look at time and energy. You will learn the basics of fluorescence polarization (FP) and fluorescence lifetime, as well as how to interpret the acronyms TRF, FRET, and TR-FRET.

e. Read–Copy–Paste–Analyze. Repeat...

Sound familiar?

In this final part of the webinar series, we look at how you can reduce the number of manual steps needed to get to your results, and remove operator error, by letting SoftMax Pro Software do the hard work for you.

Most of us have Excel on our PC and it can be a powerful analysis tool, but with SoftMax Pro Software you will have the most powerful, most published, and fully integrated microplate reader control and microplate data analysis software package available. In today's world, integrity and compliance are key, so we discuss how we support adherence to worldwide regulatory expectations and enable full FDA 21 CFR Part 11 compliance.

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