

# ELISA 101

ELISA explained: From basics  
to practical application



ELISA methods have become widely used across various fields of biology, from healthcare and food safety to cancer research and vaccine development. Whether you need to measure gluten in beer or neutralizing antibodies in serum, there is an ELISA available to provide answers to your scientific questions. Despite being first introduced over 50 years ago, ELISAs continue to be an essential tool for many researchers. This e-book provides an introduction to ELISAs and demonstrates how you can effectively use them in your own research.

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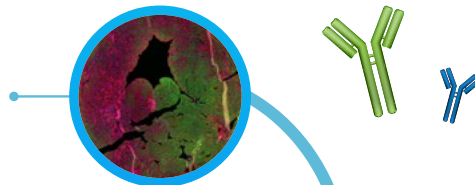
### **12 Related products and services**

# What is an ELISA?

An 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.

## History of the ELISA

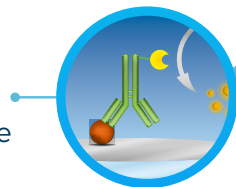
**1941**—Albert H. Coons and his colleagues are the first to label antibodies with a fluorescent dye, and use it to identify antigens in tissue sections. This method is known today as **immunofluorescence**.<sup>1</sup>



**1960**—Radioimmunoassays are described in a scientific paper by Rosalyn Sussman and Solomon Berson. However, due to radioactivity posing potential health issues, researchers were in need of a safer alternative.<sup>2</sup>



**1971**—Eva Engvall and Peter Perlman (independently) invent a method that revolutionized medicine called the **ELISA test**. The method uses antibodies to seek out the presence of hormones or viruses.<sup>3,4</sup>



Explore the origin of the ELISA and how it has evolved over the years to contribute to some of the most significant scientific breakthroughs of our time.

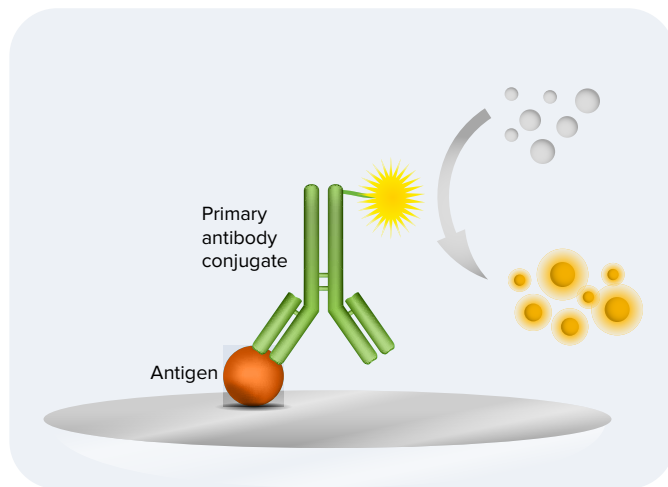
### References

1. Coons, A. H. The beginnings of immunofluorescence. *J. Immunol.* 87, 499–503 (1961).
2. Yalow, Rosalyn and Berson, Solomon. "Immunoassay of endogenous plasma insulin in man." *The Journal of Clinical Investigation.* 1960;39: 1157–75.
3. Perlmann, Peter et al. "Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G." *Immunochemistry.* 1971;8 (9): 871–4.
4. Schuurs, A. "Immunoassay using antigen—enzyme conjugates." *FEBS Letters.* 1971;15 (3): 232–236.

Read blog: The history of ELISA from creation to COVID-19 research ►

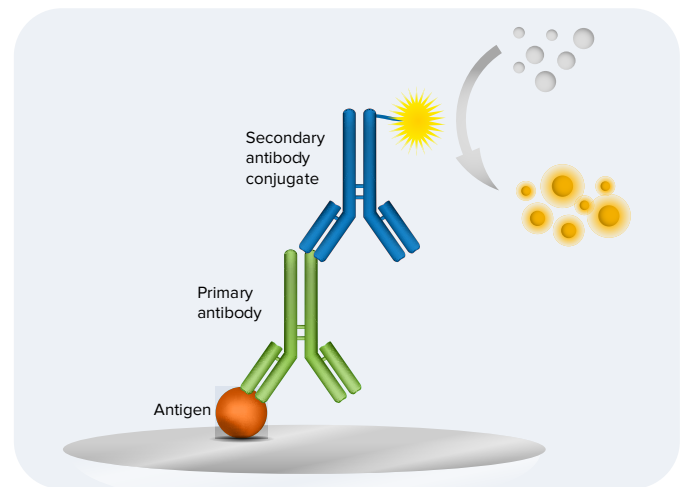
# Types of ELISA

There are four major types of ELISAs: **direct**, **indirect**, **competitive** and **sandwich**. Each type is described below with a diagram illustrating how the analytes and antibodies are used in the process.



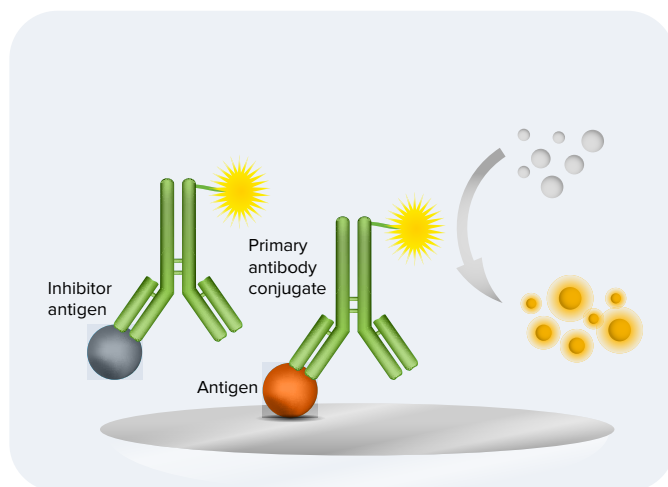
## Direct ELISA

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.



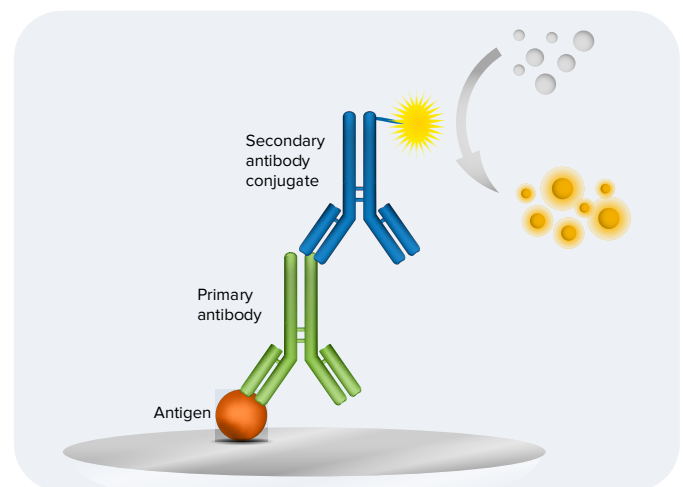
## Indirect ELISA

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 was 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

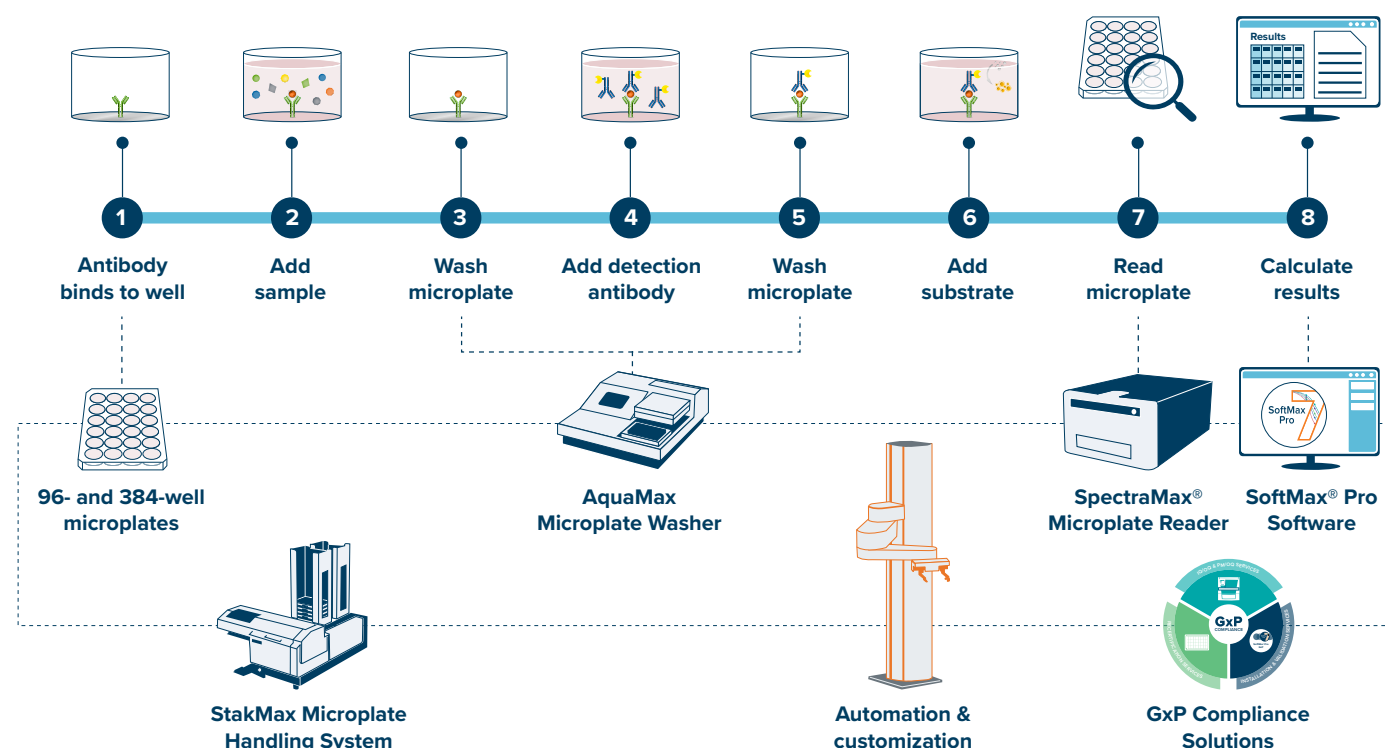
For the sandwich ELISA, two antibodies specific to two different epitopes on the target antigen are used. The capture antibody is bound to the bottom of the microplate well and binds one epitope of the antigen. The detection antibody binds to the antigen at a different epitope and is conjugated to an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

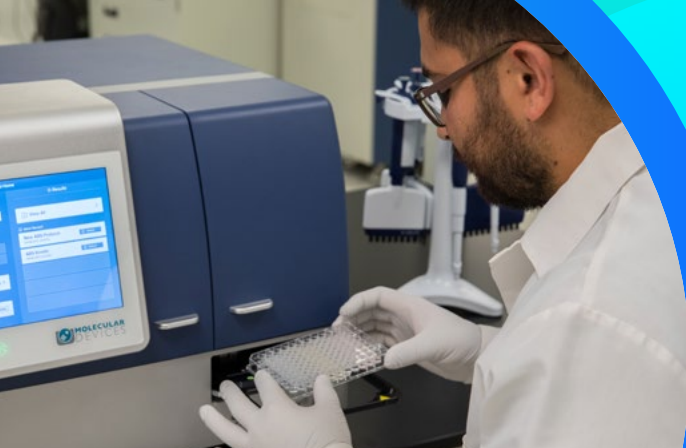
# Working with ELISAs

## Steps to run a sandwich ELISA

Most sandwich ELISAs are run in microplates, with the bottom of the plate wells serving as the solid surface to which antibodies and other reagents bind. A **microplate washer** is used to wash away non-specific material in the wells, and an **absorbance ELISA microplate reader** detects the color change produced when target antigen is present. **Plate reader software** is used to plot standard curves and calculate results.

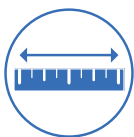
The illustration below shows a workflow for a typical sandwich ELISA:





- |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>Step 1</b> Capture antibody binds to ELISA plate wells.</p> <p><b>Step 2</b> Add sample to well – antigen within the sample binds to the capture antibody.</p> <p><b>Step 3</b> Wash microplate – Unbound material is washed away, leaving only the antigen of interest.</p> <p><b>Step 4</b> Add detection antibody – Enzyme-conjugated detection antibody binds to a second site on the antigen of interest.</p> <p><b>Step 5</b> Wash microplate – Unbound antibodies are washed away, leaving only those specific for the target of interest.</p> | <p><b>Step 6</b> Add substrate – Substrate is converted by the enzyme on the detection antibody, producing a color change.</p> <p><b>Step 7</b> Read plate – The microplate reader detects the colored reaction product and outputs optical density (OD) values.</p> <p><b>Step 8</b> Calculate results – The amount of antigen in each sample is calculated and analyzed.</p> |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

A typical ELISA protocol can be time-consuming, with multiple incubation and wash steps. CatchPoint SimpleStep ELISA kits give users a 90-minute ELISA workflow, a nearly two-thirds reduction in time compared to conventional ELISAs. Abcam's SimpleStep ELISAs reduce the time to results, but CatchPoint additionally offers better dynamic range and a more stable signal, along with sensitivity and a fast one-wash protocol.



**Extended dynamic range at the lower and upper limits**



**Stable signal for readout flexibility**



**No compromise on sensitivity**



**One-wash, 90-minute protocol**

While an ELISA is easy to set up, the assay procedure is time-consuming and labor-intensive. **Laboratory automation for high-throughput plate-based assay workflows** can help with providing walkaway time, increasing throughput, effectiveness and efficiency of the assay procedure, and reproducibility.

# Workflow of an ELISA protocol

The workflow of a typical sandwich ELISA protocol has multiple reagent addition, incubation, and wash steps. Here we've highlighted each step and the instrumentation and tools needed to conduct an ELISA including a microplate washer, absorbance ELISA plate reader and software.

## ELISA Assay Protocol Infographic



01

### ANTIBODY BINDS TO WELLS

First, the capture antibody is bound to the bottom of the microplate well.



#### ELISA Plate

Most ELISAs are run in 96- or 384- well microplates, a 96-well plate being the most common. The bottom of the microplate wells serve as the solid surface to which antibodies and other reagents attach. Microplates are often included in an ELISA kit.

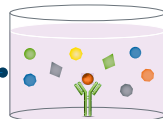
#### Want faster ELISA results?

CatchPoint SimpleStep ELISA kits produce results in just 90-minutes!

[Learn more >](#)

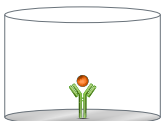


02



### ADD SAMPLE

Sample is added to the well, and antigen within the sample binds to the capture antibody.



03

### WASH MICROPLATE

Unbound material is washed away, leaving only the antigen of interest and minimizing the potential for high background signal.

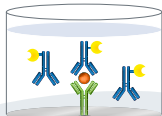


#### ELISA Plate Washer

A microplate washer is used to wash away non-specific material in the wells.

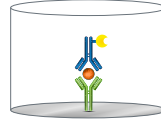
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04



### ADD DETECTION ANTIBODY

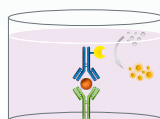
Enzyme-conjugated detection antibody binds to a second site on the antigen of interest, providing the means to detect the antigen.



05

### WASH MICROPLATE

Unbound antibodies are washed away, leaving only those specific for the target of interest and again minimizing the potential for background signal.



06

### ADD SUBSTRATE

Substrate is converted by the enzyme on the detection antibody, producing a color change, with intensity proportional to the amount of antigen present. Depending on the enzyme and substrate used, the readout can also be fluorescent or luminescent.



07

### READ PLATE

The microplate reader detects the colored reaction product and outputs optical density (OD) values that indicate how much light



#### AquaMax Microplate Washer

Aspiration and dispensing of 96- and 384-wells occur simultaneously in all wells leading to high-precision assays and faster microplate processing without mechanical plate indexing or quadrant processing.

[Watch video >](#)



#### ELISA Plate Reader

A microplate reader detects the color change produced when target antigen is present. It does so by measuring how much of the light passed through the wells of the microplate is absorbed by the material within the wells. The more antigen is present, the higher the absorbance value.

[Watch video >](#)



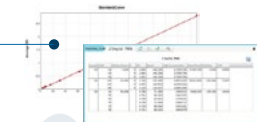
#### ELISA Plate Reader Software

Software is used to plot standard curves and calculate results from the absorbance values provided by the microplate reader.

A standard curve is run so that the amount of antigen in each sample can be accurately calculated. A preconfigured protocol, helps save time by calculating results automatically.

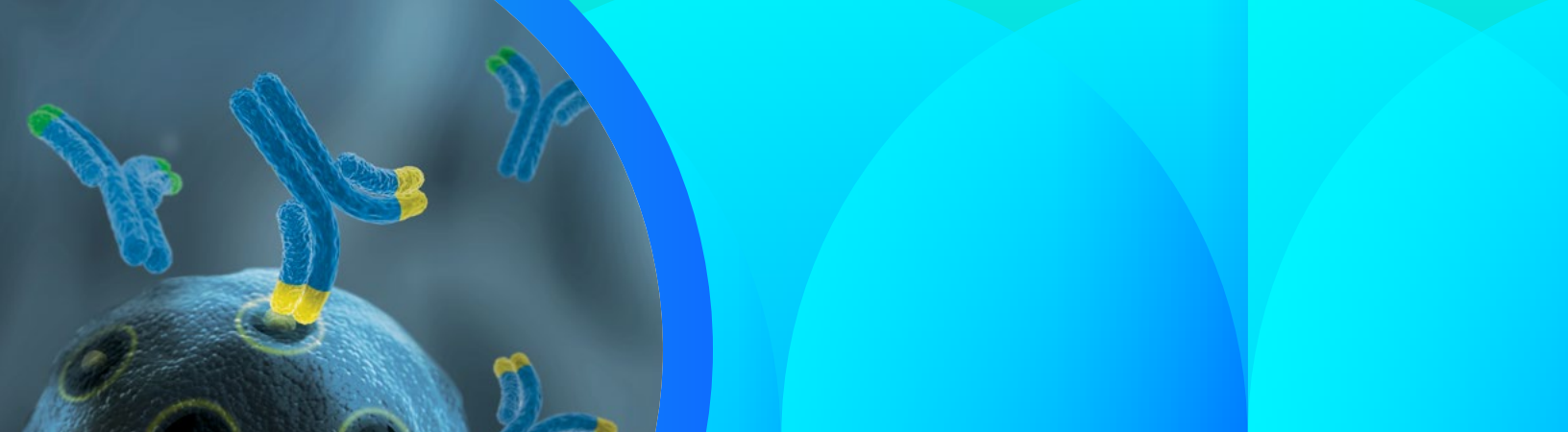
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08



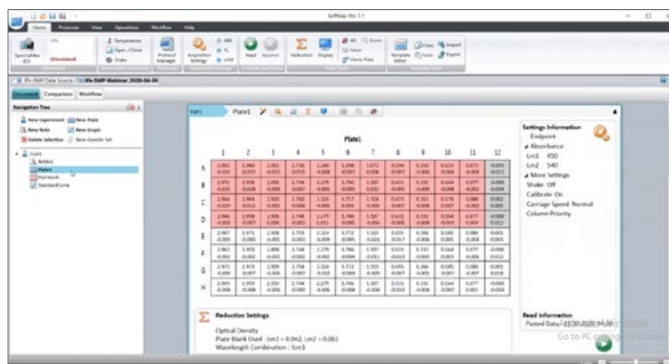
### CALCULATE RESULTS

The amount of antigen in each sample is calculated, and different samples—for example, cells subjected to different treatment conditions—can be compared.



## How to set up an ELISA and perform basic analysis

Part 1 of a four-part webinar series to introduce SoftMax® Pro 7.1.1 GxP Acquisition and Analysis Software and show how easy it is to run your assays and get results both quickly and easily.



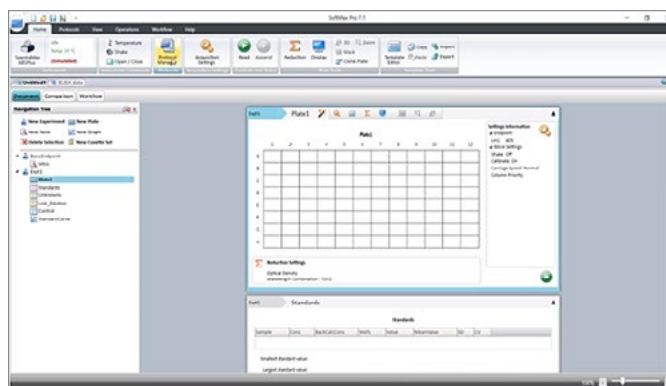
After a short introduction, you will see a live demonstration of how to use SoftMax Pro GxP Software and the SpectraMax® iD3 Multi-Mode Microplate Reader to set up and acquire data using an example ELISA. We will explain the software settings necessary to acquire data before discussing the basics of analysis. We will use an actual data set to introduce data reduction, creating a template and generating a standard curve.

[View on-demand webinar ►](#)

## How to configure an ELISA endpoint protocol

This video provides a step-by-step instruction on how to configure an ELISA endpoint protocol in SoftMax Pro Software. Using the template feature in a protocol enables rapid, automated data analysis. Curve fitting provides accurate visualization of results.

[View on-demand tutorial ►](#)



Read Application Note: Faster ELISA results with CatchPoint SimpleStep ELISA kits ►

# ELISA applications

Enzyme-linked immunosorbent assays are a commonly used analytical technique performed in many research and biotech labs. Below is a collection of application notes, research and technology related to significant ELISA assays and applications.

## Food and beverage

To ensure quality, authenticity and safety, manufacturers of foods and beverages need to perform a wide range of tests on their products. Many of these tests are based around ELISA, which are used in areas as diverse as checking for the presence of bacterial endotoxins, mycotoxins, various allergens, growth promoter hormones and antibiotics, and for meat species identification. Other absorbance tests can also be used to quantify levels of substances within foods – such as sugars, phenols, and acids.

[Learn more ►](#)



### Gluten quantitation in beer

Gliadin levels in six commercially available beers were tested to determine gluten levels with the RIDASCREEN Gliadin competitive ELISA.

[Read Application Note: Measuring gluten in beer using an ASBC-approved ELISA method ►](#)



### Detect aflatoxin in cannabis

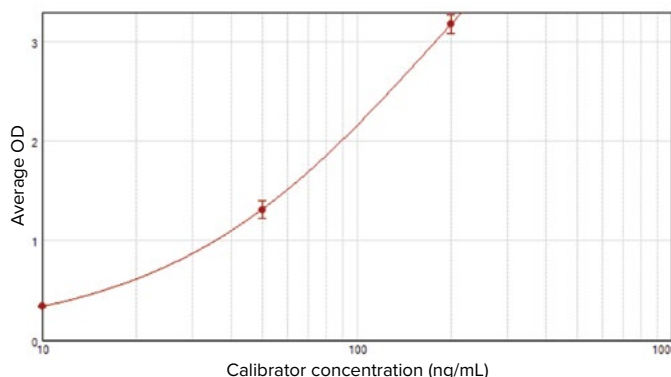
Here, we demonstrate how the AgraQuant Total Aflatoxin 4/40 ELISA kit can be used together with the SpectraMax® ABS Plus Microplate Reader and SpectraMax® iD5 Multi-Mode Microplate Reader to test for levels of aflatoxins B1, B2, G1, and G2 in one of the most common cannabis products, the flower.

[Read Application Note: Determine total aflatoxin in cannabis using an ELISA method ►](#)

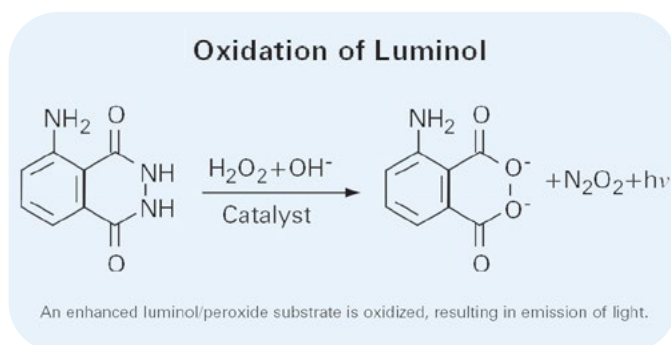
# Protein quantitation

Rapid, accurate, and frequent molecular testing is of critical importance in navigating the SARS-CoV-2 (COVID-19) pandemic. To advance our understanding of the relationship between infection and immunity, it is critical to test not only for infection, but also for antibodies.

**Read Application Note: Qualitative measurement of SARS-CoV-2 spike and nucleocapsid IgG antibodies in serum samples ►**



# Chemiluminescent VEGF ELISA



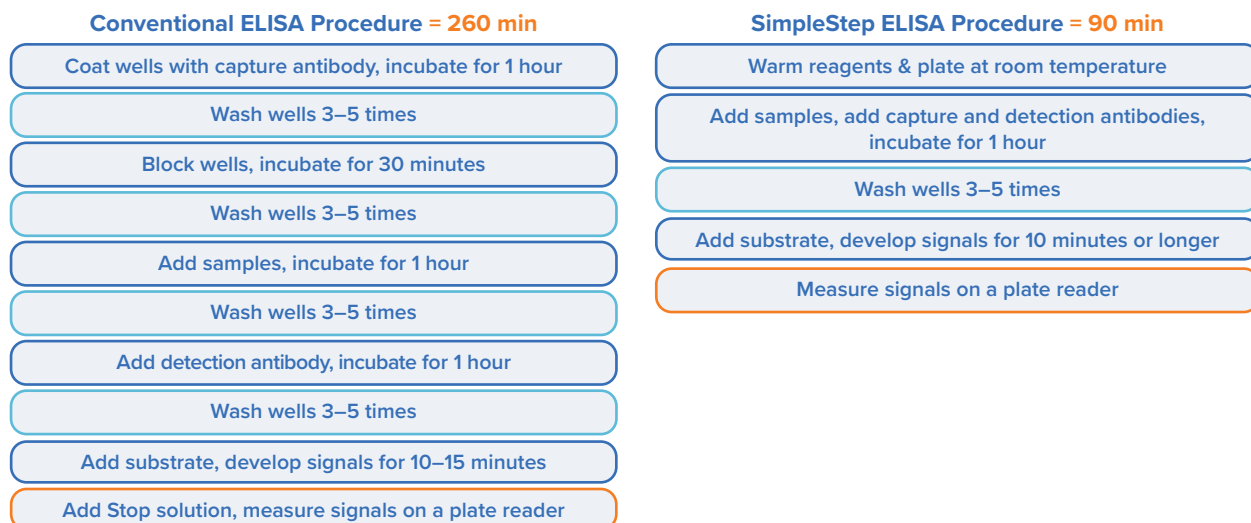
Vascular endothelial growth factors (VEGFs) are a family of secreted polypeptides that have been implicated in mammalian vascular development and in disease processes involving abnormal blood vessel growth.

**Read Application Note: Chemiluminescent VEGF ELISA Using the SpectraMax L Microplate Luminometer ►**

# Fluorescence-based detection

See how CatchPoint SimpleStep ELISA kits give users a 90-minute ELISA workflow, a nearly two-thirds reduction in the time required to achieve results compared to conventional ELISAs.

**Read Application Note: Faster ELISA results with CatchPoint SimpleStep ELISA kits ►**



# Lab automation for high-throughput ELISA assays



**ELISA** (Enzyme-linked Immunosorbent Assay) is one of the most popular quantitative methods to detect a target antigen such as a 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.

As your lab's needs expand, automating every step of an intricate workflow provides exponential value. From increased walk-away time and throughput to reduced human error and improved lab safety, automation can keep routine work on track so that scientists can focus on more critical assay development challenges.

# Related products and services

## Single-mode readers



### ABSORBANCE READERS **SpectraMax ABS and ABS Plus Absorbance ELISA Microplate Readers**

Fast and compact absorbance microplate readers for a wide range of assays without the use of filters. [Details ►](#)



### LUMINESCENT READERS **Luminescence Readers**

Single-mode microplate reader (luminometer) with luminescence detection. [Learn more ►](#)



### FLUORESCENCE READERS **Gemini™ XPS/EM Microplate Readers**

Single-mode microplate readers (fluorometers, spectrofluorometers) with fluorescence detection. [Learn more ►](#)

## Multi-mode readers



### **Multi-Mode Readers**

Absorbance, fluorescence, and luminescence detection with upgradeable high-performance capabilities [Learn more ►](#)

## Microplate reader software



### **SoftMax Pro Software**

The most published microplate reader control and data analysis software. [Details ►](#)

## GxP compliance software



### **SoftMax Pro GxP Software**

Meet regulatory guidelines in GMP/GLP labs with complete validation tools. [Learn more ►](#)

## Microplate washers



### **AquaMax Microplate Washer**

Microplate washers for biochemical and cell-based assays. [Learn more ►](#)



### **MultiWash+ Microplate Washer**

96- and 384-well automated washer. [Learn more ►](#)

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