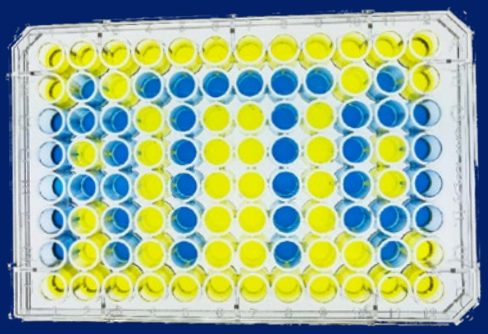
Application Note

ELISA Basic Operation



ELISA Basic Operation

January 18, 2022

Whether you were infected with coronavirus? Whether or not antibodies have been produced by vaccination? In this case, is a certain molecule (in this case, virus or antibody) present or absent in the sample (in this case, specimen, serum, etc.)? How much is contained? Immunological methods using antibodies are used to determine whether a certain molecule (in this case, a virus or antibody) is present or absent in a sample (in this case, a specimen or serum). ELISA is an effective method for detecting and quantifying specific molecules from multiple samples without the time-consuming and laborious process of Western blotting. ELISAs continue to be used as an effective method for detecting and quantifying specific molecules from multiple samples.

The unfamiliar term ELISA is an acronym for Enzyme Linked Immuno Sorbent Assay, also known as EIA (Enzyme Immunoassay) or enzyme immunoassay. It uses antibodies to detect the presence of a specific target molecule (protein, hormone, peptide, etc.) in a sample of solution containing a variety of proteins, such as blood, urine, or cell extracts.

In general, the word “Enzyme” is included in the word “Enzyme” because the detection uses enzyme-labeled antibodies such as horseradish peroxidase (HRP) and alkaline phosphatase (ALP). Enzyme-labeled antibodies are used to quantitatively measure the color development and luminescence resulting from the enzymatic reaction between the enzyme of the antibody and the substrate. For color detection of enzyme activity, a substrate whose absorbance spectrum changes with the reaction is used, and the absorbance measurement is used to quantify the amount of the target molecule. If a substrate that produces fluorescence or luminescence by enzyme reaction is used, the amount of luminescence/fluorescence is measured by a luminometer/fluorometer, etc. Radioisotope (RIA: Radio Immunoassay), chemiluminescent compound (CLIA: Chemiluminescent Immunoassay) and fluorescent dye (FIA: Fluorescent Immunoassay) are also used without enzymes.

Chromogenic reagent for HRP TMB ELISA
WSE-7145 EzELISA TMB

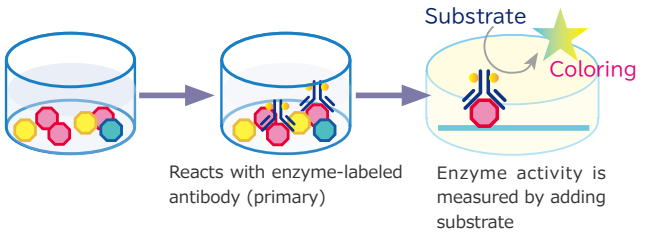
Absorbance and luminescence plate reader
WSL-2300 Phelios AL

1. types of ELISA methods

ELISA methods include direct, indirect, sandwich, and competitive methods, depending on the combination of the antibody (or antigen) to be coated on the solid phase (plate) and the labeled antibody (or labeled antigen) to be detected and the reaction method. In all of these methods, the amount of target is calculated from enzyme activity.

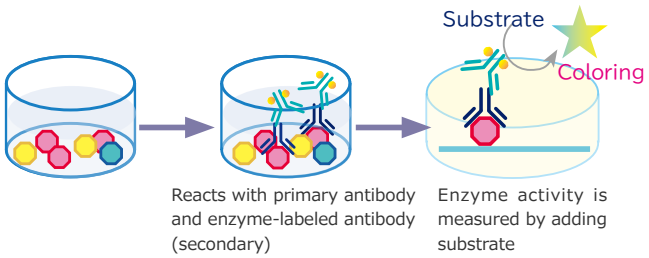
Direct ELISA

A sample (antigen) is coated on a microplate and reacted with an enzyme-labeled antibody against the target, and the enzyme activity of the labeled antibody bound to the target is measured. In some cases, antibody-coated labeled samples are used for detection.



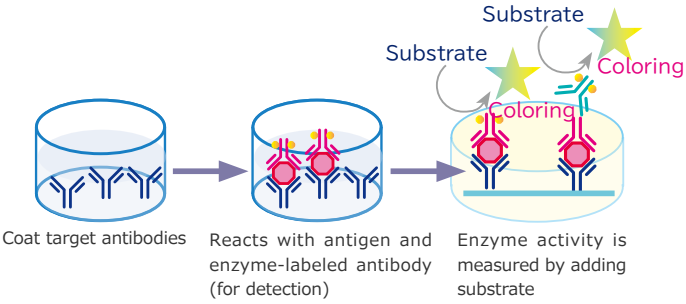
Indirect ELISA

A sample (antigen) is coated on a microplate and reacted with an antibody against the target (primary antibody), which is then reacted with an enzyme-labeled secondary antibody against the primary antibody to indirectly measure the amount of antibody bound to the target. This method does not directly label and detect primary antibodies, but indirectly detects them via enzyme-labeled secondary antibodies or enzyme-labeled avidin-biotin reaction. Compared to the direct method, the indirect method requires more reaction steps, but commercially available enzyme-labeled secondary antibodies can be used and the sensitivity is higher.



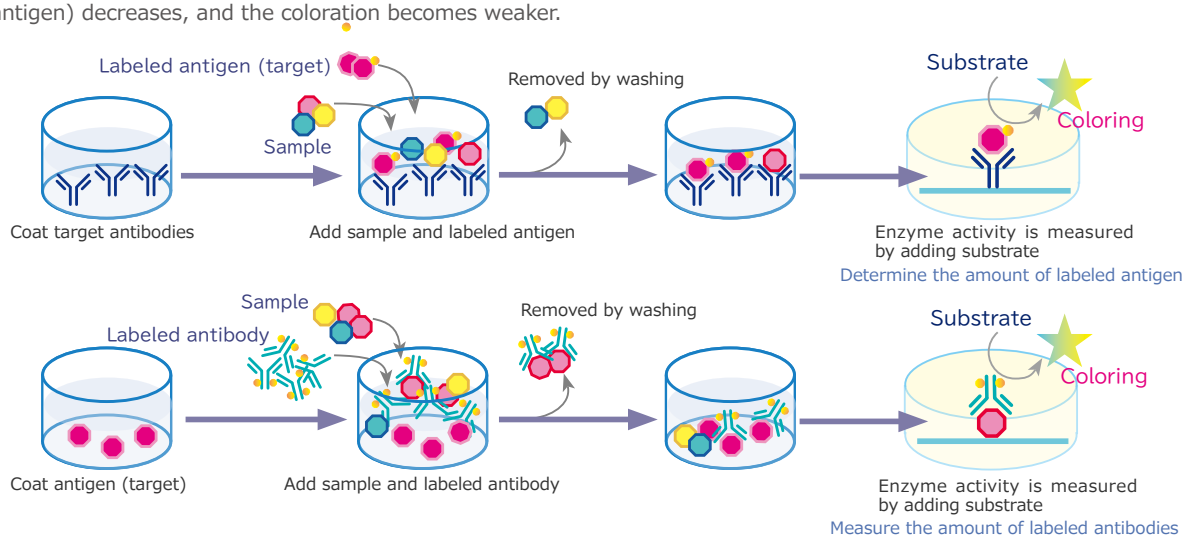
Sandwitch ELISA

The microplate is coated with an antibody against the target (for solid phase) and reacted with the sample (antigen) to be measured. Then, the antibody is reacted with another enzyme-labeled antibody against the target (for detection), and the enzymatic activity of the antibody bound to the target is measured. If an unlabeled antibody is used for detection, an enzyme-labeled secondary antibody to this antibody is used. If a biotinylated antibody is used for detection, it is detected via reaction with enzyme-labeled avidin. The epitope (antigen recognition site) of the solid-phase antibody (dark blue) and the antibody for detection (pink) are different.



Competitive ELISA

In this method, an antibody against the target (antigen) is coated on a microplate, a measurement sample and a certain amount of labeled antigen are competitively bound, the amount of labeled antigen bound to the coated antibody is detected, and the amount of the target contained in the sample is measured. As a variant method, an antigen (target) is coated on the sample, and then the sample and the labeled antibody (against the target) are added and competitively bind to each other, and the amount of the remaining labeled antibody (binding to the coated antigen) is detected. In both methods, if the sample contains a large amount of antigen, the amount of enzyme-labeled antigen that can bind to the antibody (or enzyme-labeled antibody that can bind to the antigen) decreases, and the coloration becomes weaker.



2. construction of ELISA plate

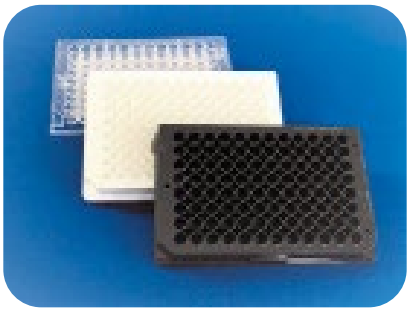
Recently, various ELISA kits are commercially available, so it is best to use them. However, if the target protein or species is unique and difficult to obtain, it is necessary to construct the kit by oneself.

Selection of detection method

Depending on the detection sensitivity and measurement range of the target protein, the labeling substance and substrate of the antibody (antigen) for detection are selected. Typical examples often used are horseradish peroxidase (HRP) and bovine small intestinal alkaline phosphatase (ALP). Other examples include luciferase, galactosidase, and acridinium derivatives. Also avoid non-water soluble substrates such as DAB and NBT/BCIP.

Selection of solid phase (plate)

The solid phases used to coat antibodies (antigens) include microplates, polystyrene beads, and magnetic beads, etc. In ELISA, microplates are used because they are convenient for the measurement of multiple samples and have high sensitivity. The solid phases for ELISA are surface-treated to change the adsorptivity of antigens and antibodies. The appropriate surface treatment differs depending on the target to be measured, so it is necessary to select a solid phase that matches the target in terms of detection sensitivity and accuracy after actual use.



In addition, ELISA plates with the appropriate color should be selected depending on the assay device and whether chromogenic (absorbance), luminescent, or fluorescent detection is to be used. In general, transparent plates are used for color detection, white plates for luminescence detection to maximize reflection and minimize self-luminescence, and black plates for fluorescence detection to minimize background during fluorescence measurement. Both types of plates are surface-coated for ELISA.

Selection of Coating Method

There are two ways to coat (insolubilize) an antibody (antigen) on a plate (solid phase surface): covalent bonding and physical adsorption. Plates with specially processed surfaces are used for covalent coating. Physical adsorption is more common, but the higher the pH of the solvent in which the protein is to be coated, the greater the amount of adsorption. If the solubilization of the protein to be coated contains special solvents or high concentrations of surfactants, they may interfere with physical adsorption, so the covalent coating method is chosen.

Selection of blocking agents

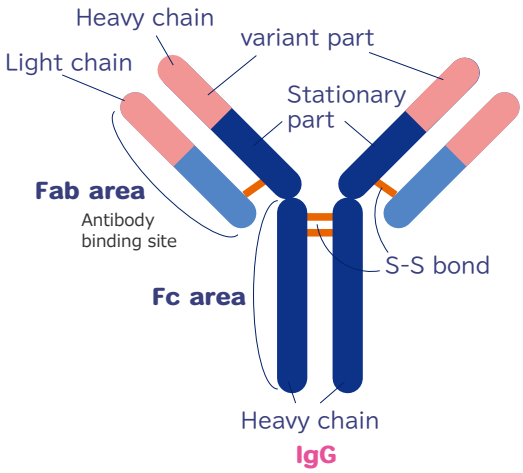
After the antibody (antigen) coats the plate, an uncoated surface remains, so this area is covered with another substance to minimize nonspecific adsorption. This operation, called blocking, is important to lower the background and improve the signal-to-noise ratio. For blocking, a solution of a protein or compound that does not denature or inhibit epitopes important to the antigen-antibody reaction and that is unrelated to the target is used. Typical blocking agents include albumin, casein, skim milk, serum, gelatin, and compounds.

ATTO blocking reagent

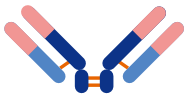
Product	EzBlock Chemi	EzBlock BSA	EzBlock CAS
type / code No.	AE-1475・2332615	AE-1476・2332616	AE-1477・2332617
main ingredient	polymer compound	BSA (albumin)	casein
Experimental Details			
For Westernblot	◎	◎	◎
For ELISA/Bead Antibody Reaction	◎	◎	○
For Immunohistochemistry/Cell Staining	○	◎	○
For diluent of antibodies	○	○	○
Reduce running costs	◎	○	○
Suppress background	○	△	◎
Prevent overblocking	◎	◎	△
For stripping	○	○	◎
Used for avidin-biotin detection system	○	○	×
Used in experiments to detect phosphorylation	◎	○	×

3. how to select antibodies

Structure of antibodies



Antibodies, also called immunoglobulins (Ig), are proteins produced by the immune response to eliminate foreign substances and are part of the body's defense mechanisms. Immunoglobulins (Ig) are divided into five classes according to structural differences: G (IgG), M (IgM), A (IgA), D (IgD), and E (IgE). It consists of two heavy chains (H chain, molecular weight of approximately 50,000) and two light chains (L chain, molecular weight of approximately 25,000), which are attached to each other by S-S bonds. The arm region where the antibody binds to the antigen is called the Fab region, which consists of the variable (V region) and stationary (C region) regions that contain the antigen recognition site (epitope). The leg portion, which mainly consists of heavy chains, is called the Fc region and is involved in effector mechanisms such as phagocytosis through binding to complement. When detecting targets in biological samples such as blood that contain complement, F(ab')2 and Fab are used, in which the Fc region of the immunoglobulin is removed by fragmentation with pepsin or papain to avoid the effects of binding to complement. Note, however, that F(ab')2 or Fab may change the specificity and binding ability of the antibody.



Pepsin digestion
$F(ab')_2$

Papain digestion
Fab

Selection of antibody

Antibodies are the most essential element in ELISA methods. Especially in sandwich and competitive methods, it is necessary to optimize not only the performance of antibodies but also the combination of antibodies. Information on compatible paired antibodies published by each antibody manufacturer can be used as a reference, or a selection can be made from paired antibodies for sandwich ELISA.

Antibody for coat (capture)

Unlike Western blotting, samples measured by ELISA are not denatured/heated by SDS or reducing agents, nor separated by electrophoresis, so the three-dimensional structure of the proteins in the sample is considered to be relatively preserved. Therefore, the epitope site that the antibody recognizes must be exposed on the surface of the three-dimensional structure of the target protein (including the complex) in order to bind to it. In addition, in the case of samples containing complement, such as serum, complement and antibody may bind nonspecifically, causing an increase in background. In such cases, use Fab or F(ab')2 without the Fc region, in which the antibody has been digested. In addition, note that additives other than antibodies (e.g., protective agents) may affect solid-phase efficiency. When purchasing antibodies, be sure to confirm the intended use, and select antibodies for “ELISA” or “IP (immunoprecipitation)” that are highly likely to bind even under non-denaturing conditions.

Antibody for detection

For the detection antibody, select an antibody that is an antibody against the surface antigen as well as the coat antibody, and that is enzyme (fluorescent)-labeled. If there is no labeled antibody for the target protein, use an enzyme-labeled secondary antibody for indirect detection, or use a commercially available labeling kit to enzyme-label the antibody for detection. Secondary antibodies should be selected with caution with regard to immunized animals, etc., to avoid cross-reactivity.

Immune animals and antibody combinations

Animals used to produce antibodies are called immunized animals. In particular, when a secondary antibody is used for indirect detection, a secondary antibody that recognizes the antibody of the immune animal that produced the antibody for detection is used. However, if the coat antibody and the detection antibody are from the same immune animal species, the secondary antibody will also bind to the coat antibody, causing the background to rise. In addition, even if antibodies derived from different immune animal species are used, they may non-specifically bind to coat and detection antibodies due to cross-reaction of the secondary antibodies; secondary antibodies should be immunoabsorbed so that they do not react with antibodies other than those of the target animal species to avoid cross-reaction. If paired antibodies are not found in different immune animal species, the antibody for detection may be biotinylated and detected with enzyme-labeled streptavidin.

Nonspecific reactions of antibodies

Due to the nonspecific reaction of the secondary antibody, it adsorbs on antigens and solid phases other than the original target antibody.

Cross-reactivity of secondary antibodies, etc.

Due to cross-reaction of secondary antibodies, the antibody reacts not only with the antibody for detection but also with the antibody for coat. The same phenomenon is likely to occur if the coat and detection antibodies are from the same species of immune animal.

Primary (antibody for coating): Mouse IgG
Secondary (antibody for detection): rabbit IgG
Tertiary (enzyme-labeled secondary antibody): anti-rabbit IgG antibody

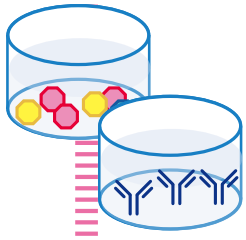
Primary: Mouse IgG
Secondary: Mouse IgG
Tertiary: Anti-mouse IgG antibody

Combination of antibodies

If the binding sites of the antibodies for coat and detection are masked, e.g., the epitopes of the antibodies for coat and detection are close, the antibodies for detection will not be able to bind.

4. experimental flow

Coat antibody(antigen)



Prepare commercially available ELISA plates.
If homemade, coat the ELISA plate with antibody (antigen).
React at room temperature for about 1 hour.

ELISA kit
ELISA kit（research only）96wel plate format
(Alzheimer's, antibody medicine, metabolic syndrome markers, and many others)

Stirring, centrifuging, and shaking equipment
WSC-2800 MyMiniVortex
WSC-2700 MyMini Spin
WSC-2400 Seesaw shaker atto

Blocking

Block the area where the antibody (antigen) was not adsorbed.
Add to the coated plate and allow to react at room temperature for approximately 1 hour.

Blocking reagent
AE-1475 EzBlock Chemi （5 × Conc.,500mL）
AE-1476 EzBlock BSA* （5 × Conc.,200mL）
AE-1477 EzBlock CAS* （5 × Conc.,200mL）
*Contains Tween-20

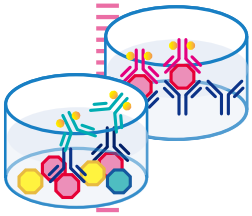
Sample preperration and reaction

Prepare a dilution series of the standard by diluting the target sample accordingly.
Add to the plate after blocking and allow to react at room temperature for approximately 1 hour.

Sample preperration/Dilution
WSE-7420 EzRIPA Lysis kit
WSE-7430 EzPBS(-)（10 × Conc.,1L）

Shaker
WSC-2400 Seesaw shaker atto

React antibody



Remove the sample, wash, and add antibody (antigen) for detection.
React at room temperature for approximately 1 hour.

Washing buffer
WSE-7430 EzPBS(-)（10 × Conc.,1L）
WSE-7230 EzTBS（10 × Conc.,1L）
WSE-7235 EzTween（10%,100mL）

Detection and measurement

Remove antibody (antigen) for detection, wash, and add substrate.
Measure luminescence/fluorescence/colorimetric values using a plate reader, etc.

Detection reagent for HRP
WSE-7145 EzELISA TMB（200mL+stop solution）
WSE-7110 EzWestLumiOne（250mL）
WSE-7120L EzWestLumi plus（250mL ×2）

Absorbance and luminescence plate reader
WSD-2300 Phelios AL (PC sold separately)

5. experimental method

This course explains how to prepare plates for sandwich ELISA, the most commonly used ELISA method, and how to measure target proteins in samples.

5-1. reagent preparation

Diluent for coat(about 20 mL is required)

50 mM Carbonate buffer/pH9.6

Add 0.15 g Na_2CO_3 and 0.29 g $NaHCO_3$ to distilled water, mix and meth up to 100 mL.

Blocking solution(about 250 mL is required)

AE-1475 EzBlock Chemi（5 × Conc.)、AE-1476 EzBlock BSA（5 × Conc.)、AE-1477 EzBlock CAS（5 × Conc.）

Dilute the stock solution 5x with distilled water; for ELISA plates, it is not necessary to add Tween 20. Please refer to the instruction manual for details.

If you make your own ELISA plate, use PBS containing 1% BSA.

Washing buffer(Can also be used for antibody dilution)

WSE-7430 EzPBS(-)（10 × Conc.)、WSE-7230 EzTBS（10 × Conc.)、WSE-7235 EzTween（10%）

Dilute the undiluted solution 10-fold with distilled water and add 0.05-0.1% Tween 20 (required for cleaning solutions) at a final concentration and mix. Please refer to the instruction manual for details.

*If you make your own, use PBS containing 0.1% Tween 20, etc.

Detection regent

WSE-7145 EzELISA TMB、WSE-7110 EzWestLumiOne、WSE-7120L EzWestLumi plus

Please refer to the respective instruction manuals for usage.

*Prepare substrates according to the labeled enzyme of the antibody to be used for detection. Note that the detection method (color, emission, fluorescence) and measurement wavelength will differ depending on the substrate.

*If you make your own substrate, use a substrate such as 0.5-1 mg/mL o-phenylenediamine/50mM phosphate-citrate buffer (pH5.0)/0.03% H_2O_2 and reaction stopper solution (such as 2-3 M H_2SO_4).

Antibodies (for coating and detection)

Antibodies for Coating (Capture) and Detection

If the antibody for detection is not labeled, the antibody is detected indirectly with a labeled antibody against the antibody for detection.

5-2. antibody coat

(1) Prepare approximately 10 mL by diluting the antibody for coat (capture) to 2-10 μg/mL with diluent for coat.

抗 The amount of antibody required per well of a 96-well ELISA plate is 100 μL. The amount of antibody adsorbed is 400~600 ng/well, depending on the surface finish of the solid phase.

(2)Add 100 μL of the diluted antibody solution in (1) per well of a 96-well plate. Incubate at room temperature for at least 1 hour or O/N incubate at 4° C.

Adsorption conditions vary depending on the antibody. Please note that detection sensitivity and measurement area may vary depending on conditions such as temperature and time.

antibody(antigen) coat

Inject slowly, making sure to place the tip of the tip on the bottom of the well. If bubbles enter the well, use a needle or similar tool to remove them.

(3) Remove the antibody for coating using an aspirator, etc., and add 250 μL/well of washing buffer (PBS-T, etc.). Remove the washing buffer and add 250 μL/well of washing buffer again. Repeat three times to wash the plate.

To prevent coated antibodies from adhering to the walls of the wells or to sites other than the bottom, remove them by aspirating with an aspirator or pipette. Wash solution can also be drained by shaking the wells upside down. Alternatively, a wash bottle filled with washing solution can be used to wash the wells by pouring a sufficient amount directly into each well. The washing solution should be drained thoroughly by dabbing the plate on a paper towel.

5-3. Blocking

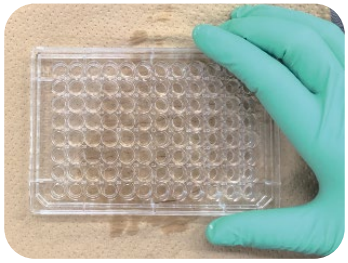
(1) Add 250 μL of blocking solution per well of a 96-well plate. Incubate at room temperature for approximately 1 hour.

Please note that detection sensitivity and measurement area may vary depending on conditions such as reaction temperature and reaction time.

(2) Remove blocking solution, add 250 μL/well of washing solution (e.g., PBS-T), and wash using the same procedure described above. Repeat the washing operation 3-4 times to wash the plate.

The blocking solution and washing solution should be drained thoroughly by patting the plate on a paper towel.

Depending on the coated antibody (antigen), the plate can be stored refrigerated in a dry state except for the blocking solution.



Drain liquid well!

For cleaning words, etc., tap the plate on a paper towel to drain the liquid thoroughly. The same applies if a plate washer is used.

5-4. sample preparation and reaction

(1) Dilute the sample to be assayed with a washing buffer or other solution as appropriate. A dilution series is also prepared by diluting a standard with a washing buffer or other solution to determine the concentration of the target protein.

(2) Add 100 µL of the dilution series of the standard and the sample per well of a 96-well plate. Incubate at room temperature for approximately 1 hour.

Please note that detection sensitivity and measurement area may vary depending on conditions such as reaction temperature and reaction time.

(3) Remove the standard and sample using an aspirator or similar device, add 250 µL/well of washing buffer (PBS-T, etc.), and wash the plate using the same procedure described above. Repeat the washing procedure 3-4 times to wash the plate.

To prevent standards and samples from adhering to other wells or well walls, remove them by aspirating with an aspirator or pipette. After washing, drain the washing solution thoroughly by dabbing the plate on a paper towel or the like.

5-5. reaction with antibody for detection

(1) Dilute the enzyme-labeled antibody for detection with washing buffer or other solution as appropriate.

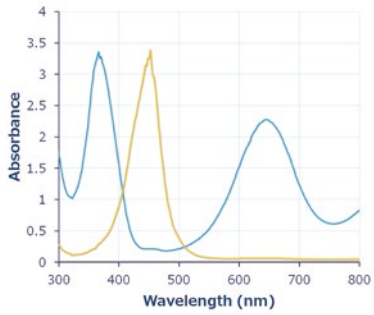
If the enzyme-labeled antibody is a polyclonal antibody or a high-titer secondary antibody (against IgG, etc.), cross-reactivity may occur or the background may be high. This may be alleviated by diluting the enzyme-labeled antibody with a blocking solution or a solution in which the blocking solution is diluted by 1/2 to 1/10 of the washing solution.

(2) Add 100 µL of enzyme-labeled antibody for detection per well of a 96-well plate. Incubate at room temperature for approximately 1 hour.

Please note that detection sensitivity and measurement area may vary depending on conditions such as reaction temperature and reaction time.

(3) Remove the enzyme-labeled antibody for detection using an aspirator, etc., add 250 µL/well of washing buffer (PBS-T, etc.), and wash the plate using the same procedure described above. Repeat the washing procedure 3-4 times to wash the plate.

To prevent enzyme-labeled antibodies from adhering to other wells or well walls, remove them by aspirating with an aspirator or pipette. After washing, the washing solution should be drained thoroughly by dabbing the plate on a paper towel.



Wavelength spectral variation of TMB substrates

When the enzyme reaction starts, the color changes to blue with maxima at 370 nm and 652 nm. The reaction stops when STOP solution is added, and the color immediately changes to yellow with a maximum wavelength at 450 nm.

5-6. detection and measurement

(1) Add 50-100 µL of substrate solution to the labeled enzyme of the antibody for detection per well of a 96-well plate.

When using luminescent or fluorescent substrates, the measurement is performed immediately after the addition of the substrate solution.

(2) Incubate at room temperature (in the dark) for approximately 5 to 30 minutes.

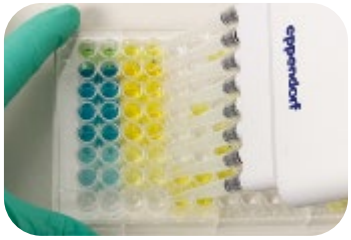
Please note that reaction conditions such as reaction time and temperature vary depending on the substrate used.。

(3) Add 50-100 µL of reaction stopping solution per well of a 96-well plate.

Please note that reaction conditions such as reaction time and temperature vary depending on the substrate used.

(4) Measure absorbance (or luminescence/fluorescence) with a plate reader or other measuring instrument.

The table below shows the measurement wavelengths, etc., of major substrates. For more details, please refer to the package insert of the enzyme or substrate to be used.



Color change of TMB substrate

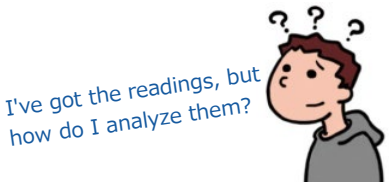
The two rows on the left side of the plate (blue to blue-green) are the coloration of the TMB substrate before reaction stoppage. Upon addition of reaction stoppage solution, the color immediately changes to yellow (third row to the right).

Enzymes and detection conditions

Enzyme	Substrate	Detection	Wave length(Reacting)	Wave length (after reaction stopped)	Detection sensitivity	
HRP	TMB	coloring : Abs	370/652 nm (blue)	450 nm (yellow)	~5 pg/well	50 pg/mL
	ABTS	coloring : Abs	650 nm (blue)	410 nm (yellow)	~250 pg/well	2.5 ng/mL
	OPD	coloring : Abs	490 nm (green)	450 nm (orange)	~7 pg/well	70 pg/mL
	Luminol	Luminescence	peak wave length : 410-425 nm		500 fg/well	5 pg/mL
	Amplex Red	Fluorescence	ex : 571 nm / em : 585 nm		~20 pg/well	2 ng/mL
ALP	PNPP	coloring : Abs	405 nm (yellow)	405 nm (yellow)	~10 ng/well	100 ng/mL
	CDP-Star	Luminescence	peak wave length : 440-460 nm		~5 pg/well	50 pg/mL
	AttoPhos	Fluorescence	ex : 440 nm / em : 560 nm		~1 pg/well	10 pg/mL

6. analysis method

ELISA methods include quantitative, semi-quantitative and qualitative analysis methods.



Quantitative

A standard curve is prepared using the measured values obtained by stepwise dilution of the standard. From the standard curve, the concentration of the target molecule in the sample is converted for quantitative analysis. In cases where there is no standard or it is difficult to prepare a standard curve, a semi-quantitative analysis method is also available, in which the increase or decrease is evaluated relative to a reference.

Qualitative

A threshold value (cutoff value) for positive/negative judgment is set, and the presence or absence of the target molecule in the sample is analyzed qualitatively as “positive” or “negative. The presence or absence of the target molecule is determined qualitatively by comparing the measured values of the sample with the negative control, positive control, or calibration control.

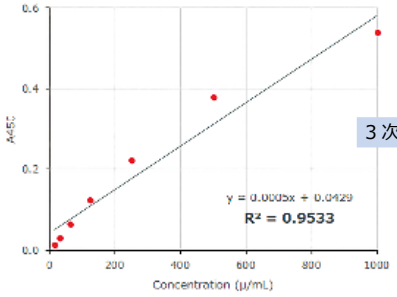
Calibration curve

Make staircase dilutions of the standard (4 or more concentrations) and measure at least 3 points for each concentration at the measurement wavelength and the reference wavelength for subtracting the plate-derived background. The values of blank samples (solvent, diluent, etc.) are also measured and subtracted from each measured value. Generally, a 4- or 5-parameter logistic is used in ELISA, but various calibration curves (standard curves) are utilized depending on the measurement range, dilution ratio of standards, antibody titer, and detection sensitivity of enzymes. The correlation coefficient (R: correlation coefficient), which is calculated to evaluate the suitability of the data, has a value in the range of -1 < R < 1. When the R2 value is 0, there is no correlation, and when it is 1 (or -1), the data line up on a straight line. If the R2 value is greater than 0.99, it can be judged to be a very good calibration curve.

Main approximations used in calibration curves

approximate curve	approximate expression
Linear approximation (linear regression)	Y=a*x+b
4-order approximation (4-order regression)	Y=a+b*x+c*x2+d*x3
3-order approximation (cubic regression)	Y=a+b*x+c*x2
logarithmic approximation	Y=a+b*log(x)
exponential approximation	Y=a*exp(b*x)
4-parameter logistic	Y=d+(a-d)/(1+(x/c)b)

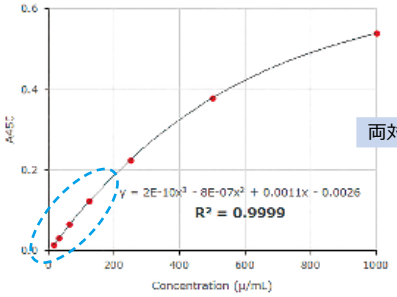
linear approximation



For measurement systems that use antigen-antibody reactions, such as ELISA, the calibration curve is generally a curve, and for a wide range of concentrations, R2 = 0.9533, which does not fit a linear approximation curve.

3 次近似に

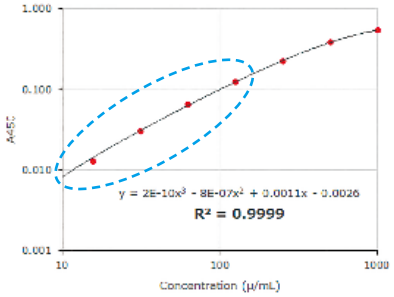
3-order approximation



A cubic approximation curve yields a well-fitted calibration curve with R2 = 0.9999. The low concentration regions are in close proximity (blue dotted enclosure) and the values cannot be read off the graph.

両対数軸に

3-order approximation



With both logarithmic axes, the curve is linear and the plots in the low-concentration region are evenly distributed (blue dotted enclosure), and the values can be read off the graph.

Measurement accuracy and sensitivity

The accuracy of the assay is affected by various factors such as the quality of standards, sample preparation methods, antibody properties, assay methods and instruments. The accuracy is evaluated in terms of the variation of measured values when the same sample is assayed in multiple wells on the same ELISA plate. The accuracy is evaluated using the Coefficient of Variation (CV), which is generally considered to be accurate if the value is less than 5%.

CV = standard deviation (SD)/mean (MEAN) x 100 (%)

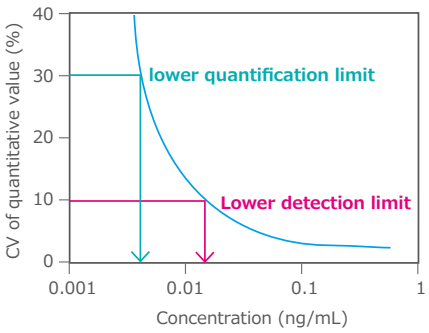
Measurement sensitivity is evaluated as the lower limit of detection or lower limit of quantitation. According to JIS standard K0462, the detection limit is defined as the amount (concentration) of a target with a CV of 30% of the quantitative value. The lower limit of detection is defined as the amount (concentration) of the target for which the CV of the quantitative value shows 30%.

Lower limit of detection (DL) = 3.3*SD of blank reading / slope of calibration curve

According to JIS standard K0462, the lower limit of quantitation is defined as the amount (concentration) of a target with a CV of 10% of the quantitation value. The lower limit of quantification is defined as the amount (concentration) of the target for which the CV of the quantification value indicates 10%.

Lower limit of quantitation (QL) = 10*SD of blank reading / slope of calibration curve

Lower Limits of Detection and Quantitation

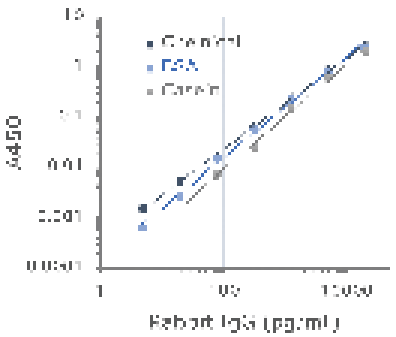


Effects of blocking agents

Direct ELISA

A 1/4 dilution series of rabbit IgG from 20 ng/mL was coated on a 96-well plate for ELISA and blocked at room temperature for 1 h. After reaction with HRP-conjugated anti-rabbit IgG antibody, the antibody was detected by a chromogenic substrate for HRP and absorbance was measured at 450 nm. The graph shows the standard curve prepared. Both blocking reagents show the same wide linear dynamic range.

Coat: Rabbit IgG (20 ng/mL~1/4 dilution series) 50µL/well 1 hour at room temperature
Blocking: EzBlock Chemi (ATTO AE-1475) 1 hour at room temperature
EzBlock BSA (ATTO AE-1476) 1 hour at room temperature
EzBlock CAS (ATTO AE-1477) Reaction at room temperature for 1 hour
Detection antibody: 1/1000 dilution HRP-conjugated anti-rabbit IgG antibody Reaction at room temperature for 1 hour
Substrate: EzELISA TMB (ATTO WSE-7145) Stopped after 10 minutes at room temperature
Analyzer: Phelios AL (ATTO WSL-2300)

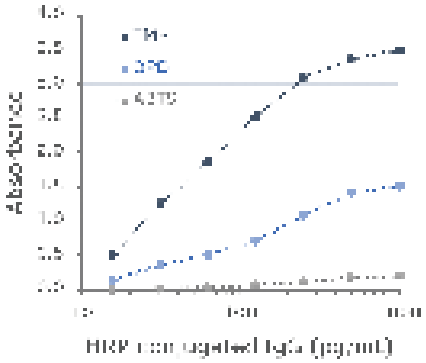


Blocking reagents and dynamic range

Difference in detection sensitivity by substrate

A 1/2 dilution series from 1000 pg/mL of HRP-labeled anti-rabbit IgG was prepared, added at 50 µL/well, and detected with three different HRP chromogenic substrates. The upper right graph shows the results of stopping the enzymatic reaction after 30 minutes and measuring the absorbance appropriate for each substrate (TMB; 450 nm, OPD; 490 nm, ABTS; 410 nm).

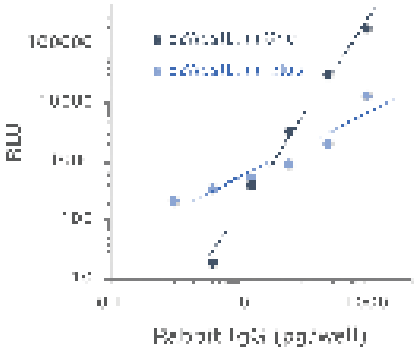
Coat: HRP-conjugated anti-rabbit IgG antibody (1 ng/mL~1/2 dilution series)
100µL/well, 1 hour at room temperature
Substrate: EzELISA TMB (ATTO WSE-7145), OPD, ABTS
Assay device: Phelios AL (ATTO WSL-2300)



Substrate type and detection sensitivity

A 1/4 dilution series of rabbit IgG from 20 ng/mL was coated on a 96-well plate for ELISA and blocked at room temperature for 1 h. After reaction with HRP-conjugated anti-rabbit IgG antibody, the antibody was detected using HRP luminescent substrate. The lower right graph shows the results of luminescence measurement and analysis with Phelios AL. In addition, EzWestLumi plus can detect low concentrations of antigen with even higher sensitivity.

Coat: Rabbit IgG (20 ng/mL ~ 1/4 dilution series) 50µL/well 1 hour reaction at room temperature
Blocking: EzBlock BSA (ATTO AE-1476), 1 hour at room temperature
Detection antibody: 1/1000 dilution HRP-conjugated anti-rabbit IgG antibody, 1 hour at room temperature
Substrate: EzWestLumiOne (ATTO WSE-7110)
EzWestLumi plus (ATTO WSE-7120)
Analyzer: Phelios AL (ATTO WSL-2300)



ELISA luminescence detection

Measurement of Human SCF in Colorectal Cancer Cell Lines by ELISA

The results of the measurement of SCF (Stem Cell Factor) levels expressed in a colorectal cancer cell line using a sandwich ELISA kit are shown. SCF is also expressed in blood stem cells and other tissues (cells), and its overexpression has been reported to be observed in malignant tumors. Colo205 and HCT116 cell lines, which are known to be highly malignant, showed higher levels of expression.

ELISA plate: Human SCF Assay Kit (ATTO 6027141)
Standard: Human SCF (3200 pg/mL ~1/2 dilution series) Reaction at room temperature for 1 hour
Detection antibody: HRP-conjugated anti-Human SCF antibody React at room temperature for 30 min.
Substrate: TMB (kit accessory)
Analyzer: Phelios AL (ATTO WSL-2300)

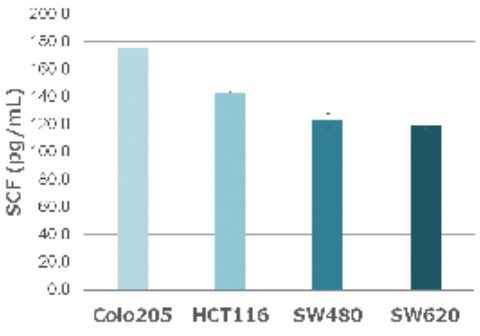


Plate reader

WSL-2300 Phelios AL

Point

Phelios AL

WSL-2300 Phelios AL is a multi-functional plate reader that can measure absorbance, kinetics, spectrum, area scan, and even luminescence in the wavelength range of 200~999nm in a lightweight and compact body. The measurement vessel is a multi-plate format compatible with 6/12/24/48/96/384-well plates. Using the “Quantitative Analysis Mode” for quantitative analysis, the troublesome creation of standard curves and concentration conversions are performed automatically at the same time as measurement, and the analysis results can be saved in Excel format. In addition, using the “Qualitative mode” for qualitative analysis, positive/negative judgment of target molecules can be made by setting a cutoff value.

Furthermore, the optional “Nano Volume Plate” enables absorbance/spectral measurement of 24 samples at once, starting from a 2 µL trace sample.

Option
Nano Volume Plate

製品情報はコチラ

Specification

Product	WSL-2300 Phelios AL
Measurement type	Absorbance（ABS）・Luminescence(LUMI)
Measurement method	ABS : Photodiode LUMI : Photomultiplier tube
Measurement mode	ABS : (1)end point (2)kinetics (3)spectra (4)area scan LUMI : (1)end point (2)kinetics Trace Absorbance : (1)end point (3)spectra
Detection sensitivity	ABS : 0 ～ 4.0 O.D. LUMI : 10^{-18} mole ATP（dynamic range ＞ 8 digit)
Measuring vessel (microplate)	6 / 12 / 24 / 48 / 96 / 384 well plate
Measurement container (Nano Volume Plate*)	3 × 8（24 sample)
Detector 1 : for ABS / wavelength	Photodiode / 200 ～ 999nm
Detector 2 : for LUMI / wavelength	Photomultiplier tube / Maximum wavelength 420nm
Wavelength Separation Method	Monochromator (1nm step)
Light source for absorbance measurement	Xenon lamp
Agitation function	Yes: 0 to 180 seconds (2-step speed)
Aplication	Measurement condition setting, measurement, data storage, and data analysis
Supported OS	Windows 10 / 11
External connection terminal	USB × 1
Dimension・weight	335mm(W) × 305mm(D) × 232mm(H)・7.0kg（AC adaptor 0.5kg)
Power Supply and Power Consumption	DC24V・40W
AC adaptor	input : AC100 ～ 240V 50/60Hz 140VA　output : DC24V 65W
Standard components	WSL-2300 Phelios AL Main unit USB cable (A-B type), AC adapter + AC cable Support plate for luminescence measurement, USB memory (attached software), Instruction manual

Easy even for beginners!

ELISA kit

This product is a research reagent. It is not intended for use in diagnosis or as an aid to diagnosis.

ELISA kits are designed to accurately, reproducibly and quantitatively measure two target proteins using a sandwich method with specific antibodies against each protein. We have a wide variety of ELISA kits for Alzheimer's disease, metabolic syndrome, cancer, etc. (For sale in Japan only)

category

抗体医療	アルツハイマー病
アレルギー	アンジオテンシン
糖尿病	炎症
肝臓・腎臓	血管
サイトカイン	細胞接着
腫瘍	ストレス
代謝	骨
免疫	老化
血液	その他

Each kit contains (1) Antibody plate, (2) Labeled antibody concentrated solution, (3) Standards, (4) Dilution buffer, (5) Lysate for labeled antibody, (6) Chromogenic substrate solution, (7) Reaction stopper solution, (8) Concentrated washing solution, and others.

Ideal for antibody reactions!

Reagents for ELISA

Point

These high-quality reagents can be used for a variety of experiments including ELISA and Western blotting. These reagents can be used for efficient experiments by eliminating the time and labor required for reagent preparation.

	code No.	type product name	
Wash buffer	2332380	WSE-7430 EzPBS(-)	Phosphate buffered saline solution (10×concentration, 1L)
	2332625	WSE-7230 EzTBS	Tris-buffered saline solution (10 x concentration, 1 L)
	2332626	WSE-7235 EzTween	10% Tween solution (100 x concentration, 100 mL)
Blocking reagent	2332617	AE-1477 EzBlock CAS	Casein-containing blocking reagent (5 x concentration, 200 mL)
	2332616	AE-1476 EzBlock BSA	Blocking reagent containing BSA (5 x concentration, 200 mL)
	2332615	AE-1475 EzBlock Chemi	Non-protein-based blocking reagent (5 x concentration, 500 mL)
Chromogenic detection reagent	2332458	WSE-7145 EzELISA TMB	Chromogenic reagent for HRP Set of TMB solution and reaction stopper (200 mL each)
Chemiluminescence detection reagent	2332632	WSE-7110 EzWestLumiOne	One liquid system Luminescent substrate for HRP (250 mL each)
	2332637	WSE-7120S EzWestLumi plus	Two-component system Luminescent substrate for high-sensitivity HRP (50 mL each)
	2332638	WSE-7120L EzWestLumi plus	Two-component system, luminescent substrate for high-sensitivity HRP, large-volume version (250 mL each)
Protein Extraction Kit	2332336	WSE-7420 EzRIPA Lysis kit	Protein extracts from animal cells and tissues (including protease inhibitor/phosphatase inhibitor, 100 mL)
	2332339	WSE-7423 EzBactYeastCrusher	Protein extract from E. coli and yeast (including protease inhibitor/ DNAse, 100 mL)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