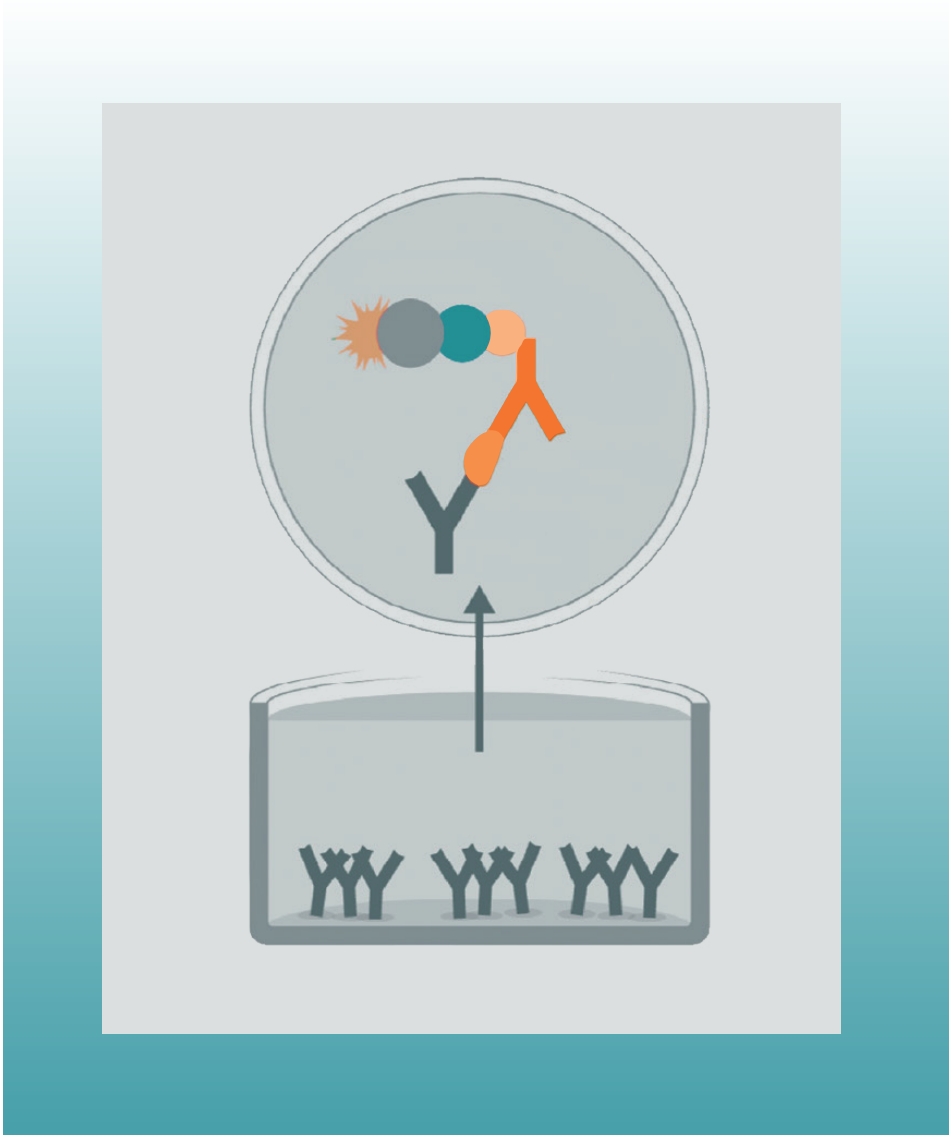
Enzyme-linked immunosorbent assay

ELISA guide

abcam 推出的 ELISA 使用指南，讓您在 ELISA 遇到的實驗問題能找到解答。



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Types of ELISA

ELISA 分析可以有不同的格式，每種都有其優缺點，下圖說明了 4 種主要不同類型的 ELISA。

Direct ELISA

抗原被固定在多孔盤表面，並使用特定於抗原的抗體進行偵測，
該抗體直接連接到 HRP 或其他偵測分子。

Indirect ELISA

類似於直接 ELISA 分析，抗原被固定在多孔盤表面。
然而，檢測需要進行兩個步驟，一個特定於抗原的一級抗體與目標結合，
而對應一級抗體主宿主物種的標記二級抗體與一級抗體結合以進行檢測。

Sandwich ELISA

三明治 ELISA 是最常見的形式，需要兩種針對抗原不同表位的抗體。
這兩種抗體通常為匹配的抗體對，其中一種抗體被塗層在多孔盤表面並用作捕獲抗體，
以促進抗原的固定，另一種抗體則與抗原結合進行檢測。

Competitive ELISA

這種測定法也被稱為抑制 ELISA 或競爭型免疫測定法，通過檢測信號干擾來測量抗原的濃度。
樣本抗原與帶有酵素之抗原競爭結合到特定量的標記抗體上，
根據樣本中抗原的量，會有更多或更少的游離抗體可用於結合帶有酵素之抗原，
這意味著樣本中的抗原越多，檢測到的帶有酵素之抗原就越少，信號就越弱，反而亦之。

Choose the right ELISA kit

仔細評估測定性能力是選擇每組 ELISA 試劑重要的一環，包括靈敏度 (sensitivity)、動態範圍 (dynamic range) 和精確度，其他參數能夠對不同 ELISA 性能有更好的預測性。了解不同的參數的相關參考數值，以幫助您找到適合樣本的ELISA試劑。

Parameter	Acceptance criteria
Sensitivity	取決於不同的目標蛋白*
Dynamic range	
CV (%) for intra-assay precision	≤ 10%
CV (%) for inter-assay precision	≤ 15%
Specificity	確認與高度同源蛋白質的反應性
% Recovery	≥ 80%
Linearity of dilution	≤ 20% 差異相比於未稀釋檢體

*了解檢體中預期的目標蛋白質水平非常重要，需確保所使用的 ELISA 試劑的靈敏度和動態範圍是合適的。對於目標蛋白質濃度較高的樣本，可以進行稀釋，使原始信號處於測定的動態範圍之內。

ELISA Sample preapration

請參考試劑附帶的說明書，以獲取有關檢體處理和相容樣本類型的產品特定細節，最佳樣本準備程序將取決於所測試的目標和分析方法，本節將提供樣本製備方法供您參考。

Sample preparation methods

Cell culture supernatant 細胞培養上清液

- Pipette cell culture media into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4°C.
- Immediately aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

Cell extract 細胞萃取物

- Place tissue culture plates on ice.
- Aspirate medium and gently wash cells once with ice-cold PBS.
- Aspirate PBS and add 0.5 mL complete extraction buffer per 100 mm plate.
- Scrape cells to collect in tilted plate and remove to pre-chilled tube.
- Vortex briefly and incubate on ice for 15–30 min.
- Centrifuge at 13,000 rpm for 10 min at 4°C to pellet insoluble contents.
- Aliquot supernatant (this is the soluble cell extract) to clean, chilled tubes on ice and store samples at -80°C. Minimize freeze/thaw cycles.

Conditioned medium 條件培養基

- Place cells in complete (serum-containing) growth medium and allow cells to proliferate to desired level of confluence.
- Remove growth medium and wash very gently with a few mL of warm PBS. Repeat wash step.
- Remove last PBS wash and gently add serum free growth medium.
- Incubate 1–2 days.
- Pipette medium into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4°C.
- Immediately aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

Milk 牛奶

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

Plasma 血漿

- Collect whole blood into anti-coagulant containing tube or add 0.1 M sodium citrate to 1/10 final volume.
- Centrifuge at 3,000 rpm for 10 min at 4°C.
- Immediately aliquot supernatant (plasma) and store samples at -80°C. Minimize freeze/thaw cycles.

Urine 尿液

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

Saliva 唾液

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

Serum 血清

- Collect whole blood in untreated test tube or, for example, an anti-coagulant free tube.
- Incubate undisturbed at room temperature for 20 min.
- Centrifuge at 3,000 rpm for 10 min at 4°C.
- Immediately aliquot supernatant (serum) and store samples at -80°C. Minimize freeze/thaw cycles.

Tissue extract 組織萃取物

- Dissect the tissue of interest with clean tools, preferably on ice and as quickly as possible to prevent degradation by proteases.
- Place the tissue in round bottom microfuge tubes and immerse in liquid nitrogen to snap freeze. Store samples at -80°C for later use or keep on ice for immediate homogenization.
- For a ~5 mg piece of tissue, add ~300 µL complete extraction buffer (see cell/tissue extraction buffer recipe) to the tube and homogenize with an electric homogenizer.
- Rinse the blade twice using 300 µL complete extraction buffer for each rinse, then maintain constant agitation for 2 h at 4°C (eg place on an orbital shaker in the cold room).
- Centrifuge for 20 min at 13,000 rpm at 4°C. Place on ice, aliquot supernatant (this is the soluble protein extract) to a fresh, chilled tube and store samples at -80°C. Minimize freeze/thaw cycles

Control samples required for ELISA

設計控制組有助於精確區分真正的陽性結果與潛在的偽陽性，可有效幫助實驗數據的驗證。
以下為 ELISA 時應使用的各種控制樣品類型：

Positive control

可使用含有所檢測蛋白的內源性可溶性樣品，或使用已知含有所使用抗體的抗原序列的純化蛋白或生肽。
即使樣本為陰性，正控制的陽性結果也可代表實驗正常，確認任何陰性結果的有效性。

We recommend checking the antibody datasheet, which will often provide a suggested positive control.
If no control is suggested, we recommend the following:

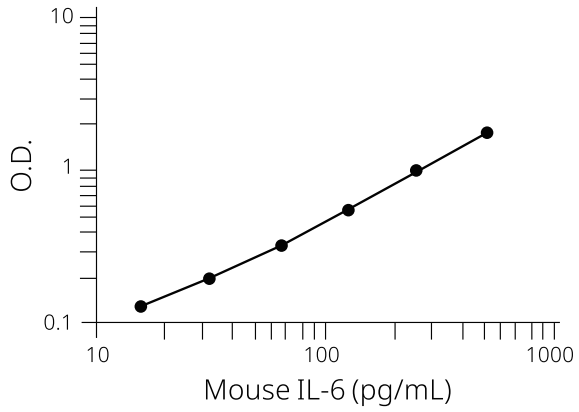
- Check to see if there are any Abreviews® for the antibody. Any tissues, cells, or lysates that have been used successfully can be considered a suitable positive control.
- Try looking at the Swiss-Prot or Omnigene database links on the datasheet. These databases will often have a list of tissues that the protein is expressed in. These can also be considered suitable positive controls.
- Check the GeneCards entry for the protein. This will usually provide you with relative levels of expression in various tissues.
- If you still have difficulty finding a suitable control, we recommend doing a quick literature search on PubMed to see which tissues and cells express the protein of interest.

Negative control

使用無法檢測該蛋白的樣品，建議在每次試驗中設計負控制組，可驗證非特異性結合和假陽性結果。

Standard Curve

使用已知濃度的目標蛋白可做出標準曲線。
不良的標準曲線代表抗體未能正確結合，或無法成功捕捉蛋白，趨勢線的 R^2 值通常應大於0.99。



例: 使用 Mouse IL-6 ELISA (ab46100) 做出的標準曲線，濃度範圍從 15.6 到 500 pg/mL。

Standard in sample matrix (spike control) control

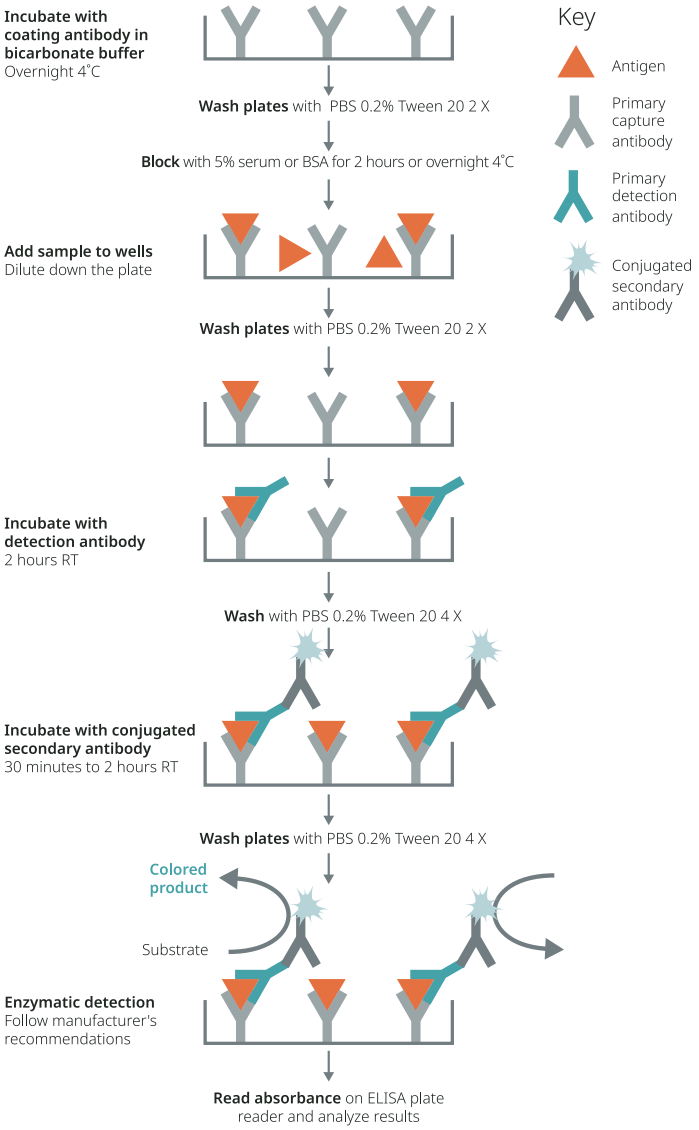
使用血清為檢體時應設計兩組標準曲線，一組為正常稀釋，另一組則使用血清進行稀釋，兩者可以進行比較確保血清中的其他蛋白不會對標準曲線產生影響，也稱Spike control。確認目標蛋白在添加到基質 (matrix) 後是否可被正確測定，可接受的範圍在 80-120% 之間。

Endogenous positive control

如果您正在測試一個重組蛋白樣本，應包括一個內源性正控制組。
對於抗體檢測重組蛋白質存在一些問題，因為重組蛋白可能與內源性的天然形式不同，可能導致抗體無法辨認抗原表位，更須注意重組蛋白須包含在您所使用的抗原辨認序列中。內源性正控制組對於驗證結果以及顯示試劑是否在過程中反應正常來說相當重要。

Sandwich ELISA protocol

A sandwich ELISA measures antigen between two layers of antibodies (capture and detection antibody). The target antigen must contain at least two antigenic sites capable of binding to antibodies



ELISA analysis

ELISA standard curve

標準曲線或校準曲線是定量 ELISA 的一個要素，可計算樣本中檢體的濃度。
通常以下列幾個曲線模型來製作標準曲線:

- **Linear plot: y=a+bx**

  線性回歸是最簡單的回歸模型，也是最基本的曲線回歸分析方法，然而線性回歸通常會壓縮到低濃度的點導致濃度低估的情形。

- **Semi-log plot: y = a log(x) + b**

  半對數擬合是將濃度值取對數，然後與對應讀值進行線性回歸。
  這種方法常用於當濃度隨著讀值的增加或減少，變化比讀值變化更大的情況下使用。

- **Log-log plot: log(y) = a log(x) + b**

  對於濃度的低至中等範圍提供良好的線性，但較高範圍的部分往往會失去線性。

- **4- or 5-parameter logistic (4PL or 5PL) curves**

  因考慮了其他參數如最大值 (maximum) 和最小值 (minimum)，需要進行更複雜的計算。
  4PL 假設在反曲點 (inflection point) 周圍是對稱的而 5PL 則不考慮，通常對免疫分析更為適用。

通常建議以 4-PL 與 5-PL 來進行數據計算，再來可以選擇以semi-log 或是 log/log 進行作圖。

Scan here to download the full guide!

ELISA troubleshooting tips

Poor standard curve

Cause	Solution
Improper standard solution	Confirm dilutions are made correctly.
Standard improperly reconstituted	Briefly spin vial before opening; inspect for undissolved material after reconstituting.
Standard degraded	Store and handle standard as recommended.
Curve doesn't fit scale	Try plotting using different scales, eg log-log, 5 parameter logistic curve fit.
Pipetting error	Use calibrated pipettes and proper pipetting technique.

No signal

Cause	Solution
Incubation time too short	Incubate samples overnight at 4°C or follow the manufacturer guidelines.
Target present below detection limits of assay	Decrease dilution factor or concentrate samples.
Incompatible sample type	Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect a positive control.
Recognition of epitope impeded by adsorption to plate	To enhance detection of a peptide by direct or indirect ELISA, conjugate peptide to a large carrier protein before coating onto the microtiter plate.
Assay buffer compatibility	Ensure assay buffer is compatible with target of interest (eg enzymatic activity retained, protein interactions retained).
Not enough detection reagent	Increase concentration or amount of detection reagent, following manufacturer guidelines.
Sample prepared incorrectly	Ensure proper sample preparation/dilution. Samples may be incompatible with microtiter plate assay format.
Insufficient antibody	Try different concentrations/dilutions of antibody.

Cause	Solution
Incubation temperature too low	Ensure the incubations are carried out at the correct temperature. All reagents including plate should be at room temperature or as recommended by the manufacturer before proceeding.
Incorrect wavelength	Verify the wavelength and read plate again.
Plate washings too vigorous	Check and ensure correct pressure in the automatic wash system. Pipette wash buffer gently if washes are done manually.
Wells dried out	Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations.
Slow color development of enzymatic reaction	Prepare substrate solution immediately before use. Ensure the stock solution has not expired and is not contaminated. Allow longer incubation.

Large coefficient of variation (CV)

Cause	Solution
Bubbles in wells	Ensure no bubbles are present prior to reading plate.
Wells not washed equally/thoroughly	Check that all ports of the plate washer are unobstructed. Wash wells as recommended.
Incomplete reagent mixing	Ensure all reagents are mixed thoroughly.
Inconsistent pipetting	Use calibrated pipettes and proper technique to ensure accurate pipetting.
Edge effects	Ensure the plate and all reagents are at room temperature.
Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg minimize freeze/thaw cycles).

High background

Cause	Solution
Wells are insufficiently washed	Wash wells as per protocol recommendations.
Contaminated wash buffer	Prepare fresh water buffer.
Too much detection reagent	Ensure the reagent has been diluted properly or decrease the recommended concentration of detection reagent.
Blocking buffer ineffective (eg detection reagent binds blocker; wells not completely blocked)	Try different blocking reagent and/or add blocking reagent to wash buffer.
Salt concentration of incubation/wash buffers	Increasing salt concentrations may reduce non-specific and/or weak off-target interactions.
Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution.
Non-specific binding of antibody	Use suitable blocking buffers, eg BSA or 5-10% normal serum - species same as primary antibody if using a directly conjugated detection antibody or same as secondary if using conjugated secondary. Ensure wells are pre-processed to prevent non-specific attachment.
High antibody concentration	Try different dilutions for optimal results.
Substrate incubation carried out in light	Substrate incubations should be carried out in the dark or as recommended by manufacturer.
Precipitate formed in wells upon substrate addition	Increase dilution factor of sample or decrease concentration of substrate.
Dirty plate	Clean the plate bottom.

Low sensitivity

Cause	Solution
Improper storage of ELISA kit	Store all reagents as recommended. Please note that all reagents may not have identical storage requirements.
Not enough target	Concentrate sample or reduce sample dilution.
Inactive detection reagent	Ensure reporter enzyme/fluorophore has the expected activity.
Plate reader settings incorrect	Ensure plate reader is set to read the correct absorbance wavelength or excitation/emission wavelengths for fluorescent detection.
Assay format not sensitive enough	Switch to a more sensitive detection system (eg colorimetric to chemiluminescence/ fluorescence). Switch to a more sensitive assay type (eg direct ELISA to sandwich ELISA). Lengthen incubation times or increase temperature.
Target poorly adsorbs to microtiter plate	Covalently link target to microtiter plate.
Not enough substrate	Add more substrate.
Incompatible sample type (eg serum vs cell extract)	Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect as a positive control.
Interfering buffers or sample ingredients	Check reagents for any interfering chemicals. For example, sodium azide in antibodies inhibit HRP enzyme and EDTA used as anticoagulent for plasma collection inhibits enzymatic reactions.
Mixing or substituting reagents from different kits	Avoid mixing components from different kits.

Matrix effect

進行血漿和血清的定量分析有時會出現 (matrix effect) 基質效應。

基質效應可包括來自多種基質所組成，如：內源性生物組分之間的相互作用，如磷脂、碳水化合物和內源代謝產物（膽紅素）之間的作用；或者帶測物與基質之間的相互作用，如：與血漿蛋白共價結合，種種都可能導致數據的誤判。

透過將檢體稀釋 2-5 倍可以減少基質效應，須注意在稀釋檢體時使用與標準曲線相同的稀釋溶液。

Everything you need to perform your ELISA experiments

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