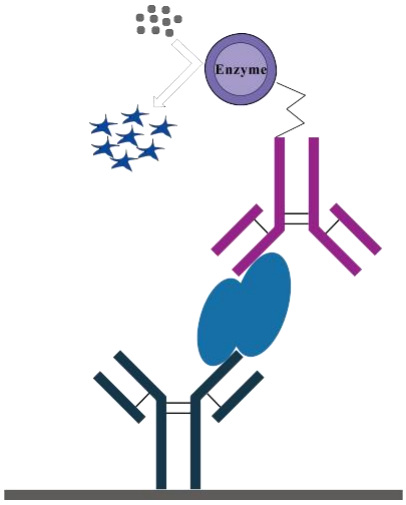
Technical Guides

Instruction Manuals
Common Questions

Principle of ELISA

1 *Sandwich ELISA*

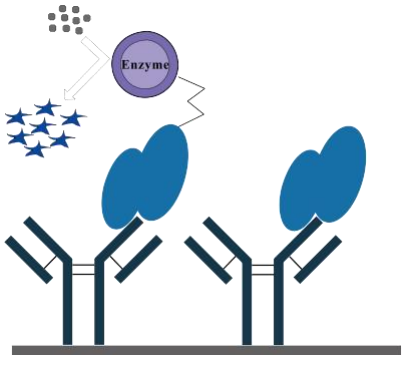
IL-1 ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with a monoclonal antibody specific for IL-1. Standards or samples are then added to the microtiter plate wells and IL-1 if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of IL-1 present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for IL-1 are added to each well to "sandwich" the IL-1 immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-1 and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The IL-1concentration in each sample is interpolated from this standard curve.



Sandwich ELISA

2 *Competitive ELISA*

Cortisol ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-Cortisol antibody and an Cortisol-HRP conjugate. The assay sample and buffer are incubated together with Cortisol-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the Cortisol concentration since Cortisol from samples and Cortisol-HRP conjugate compete for the anti-Cortisol antibody binding site. Since the number of sites is limited, as more sites are occupied by Cortisol from the sample, fewer sites are left to bind Cortisol-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards . The Cortisol concentration in each sample is interpolated from this standard curve.



Competitive ELISA

Instruction Manuals

How to prepare samples

Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000 × g (or 3000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C..

Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed 300-500mg before homogenization. Minced the tissues to small pieces and homogenized them in 500ul of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm).Collect the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

Cell lysates-Cells should be lysed according to the following directions

- Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
- Wash cells three times in PBS. As for the collection of the samples, the amount of cells should be no less than 10^8 in 200ul PBS.
- Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
- Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8 °C to remove cellular debris.
- Assay immediately or store samples at -20°C or -80°C.

Cell culture supernatants and other body fluids

Centrifuge cell culture media at 1000 × g (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

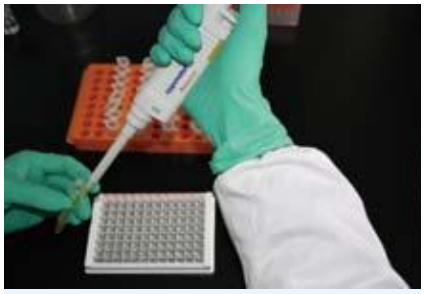
NOTE

1. Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.
2. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
3. Samples containing a visible precipitate must be clarified prior to use in the assay. Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
4. Do not use heat-treated specimens.

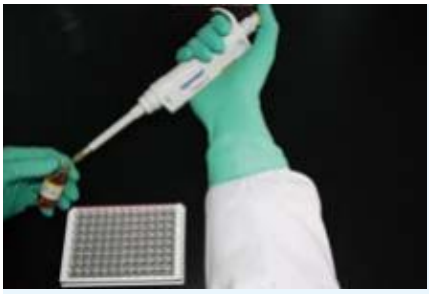
Instruction Manuals

Assay Procedure Summary

1 Bring all reagents and samples to room temperature before use.

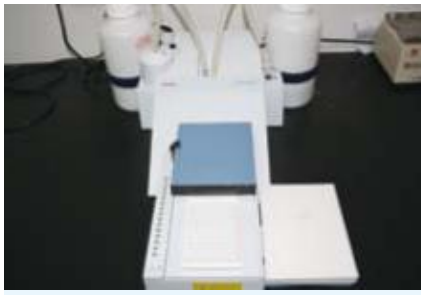


2 Add Standards or Samples to wells in pre-coated microtiter plate.



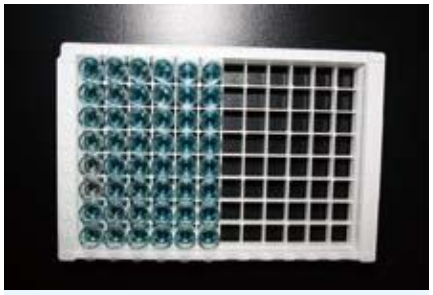
3 Add Enzyme Conjugate to each well. Mix well.

4 Cover and incubate the plate for 1 hour at 37℃.

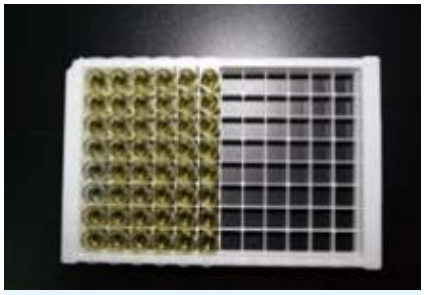


5 Wash the microtiter plate using wash machine for five times.

6 Add Substrate A and Substrate B to each well, subsequently.



7 Cover and incubate for 10-15 minutes at 20-25℃. (Avoid sunlight).



8 Add Stop Solution to each well. Mix well.

9 Determine the optical density at 450 nm using a microplate reader.

Problem	Possible Cause	Solution
No Signal	Reagents added in incorrect order, or incorrectly prepared	Repeat reagent addtion correctly according to the manual
	Contamination of HRP with azide	Use fresh reagents
	Standard has gone bad (if thereis a signal in the sample wells)	Check that standard was handled according to the manual
	Buffer containing FCS used to reconstitute antibodies	Requalify your reagents of choice
	Buffers contaminated	Make fresh buffers
Too much signal	Insufficient washing or washing step skipped – unbound peroxidase remaining	Wash thoroughly according to the manual
	Substrate Solution mixed too early and turned blue	Substrate Solution should be mixed and used immediately
	Too much streptavidin-HRP	Check dilution, titrate ifnecessary
	Plate sealers or reagent reservoirs reused, resulting inpresence of residual HRP. This will turn the TMB blue nonspecifically	Use fresh plate sealer and reagent reservoir for each step
	Buffers contaminated with metals or HRP	Make fresh buffers
Poor Duplicates	Plate sealer reused	Use a fresh plate sealer for eachstep
	No plate sealers used	Use plate sealers
	Buffers contaminated	Make fresh buffers
	Insufficient washing	Wash thoroughly according to the manual. If using an automatic platewasher, check that all ports are clean and free of obstructions
Poor assay to assay reproducibility	Variations in incubation temperature	Adhere to recommended incubation temperature. Avoid incubating plates in areas where enviromental conditions vary
	Variations in protocol	Adhere to the same protocol from run to run
	Plate sealer reused, resulting in presence of residual HRP which will turn the TMB blue	Use fresh plate sealer for eachstep
	Buffers contaminated	Make fresh buffers
No signal when a signal is expected, but standard curve looks fine	No cytokine in sample	Use internal controls. Repeat experiment, reconsider experimental parameters
	Sample matrix is masking detection	Please use only undiluted or concentrated samples
Samples are reading too high, but standard curve looks fine	Samples contain cytokine levels above assay range	Dilute samples and run again
Very low readings across the plate	Incorrect wave lengths	Check filters/reader
	Insufficient development time	Increase development time
	Buffer containing FCS used to reconstitute antibodies	Requalify your reagents of choice
Green color develops uponaddition of stop solution when usingstreptavidin-HRP	Reagents not mixed well enough in wells	Tap plate
Edge Effects	Uneven temperatures around work surface	Avoid incubating plates in areas where environmental conditions vary. Use plate sealers
Drift	Interrupted assay set-up	Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay
	Reagents not at roomtemperature	Ensure that all reagents are atroom temperature beforepipetting into the wells unlessotherwise instructed in theantibody inserts