Sandwich Elisa General Protocol

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

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Store: 4 ^{o}C

Safety: Stop solution contains acid. Avoid all contact and inhalation.

BACKGROUND

These ELISA kits are aimed at detecting soluble factors in serum, blood, plasma and other related tissues and employs the double-antibody sandwich method. Briefly, the provided 96 well plate is coated with a monoclonal antibody selective for the antigen of interest. Serum or blood samples are added to the wells. In parallel, the provided standard is added to other wells in a dilution series in the provided dilution buffer. After an incubation period, unbound proteins in the wells are washed. Another provided antibody also to the antigen and conjugated with horse radish peroxidase (HRP) is added, followed by additional washes of unbound antibody and the addition of chromogens. The reaction is stopped by the addition of a sulfuric acid stop solution. Absorbance is measured in a microplate reader, which based on the standard, quantifies the amount of soluble factor in the assay sample.

Table 1. Materials supplied.

1	Standard (960ng/L)	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent	3ml	8	Chromogen Solution B	6ml
3	Micro ELISA strip plate	12well×8strips	9	Stop Solution	6ml
4	HRP-Conjugate Reagent	6ml	10	Instruction	1
5	30× wash solution	20ml	11	Closure plate membrane	2
6	Sample diluent	6ml	12	Sealed bags	1

Materials required but not supplied

1. 37 ^{o}C incubator
2. Standard microplate reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Important notes in preparation of the assay

1. The kit should be equilibrated to room temperature for 30 min after removal form 4 ^{o}C storage.
2. Store open microplates in a seal zip lock bag
3. Standards should be used in duplicate.
4. If the test reading is excessively high, you may wish to dilute your sample in PBS and re-perform the assay to assure readings are not at saturation. When calculating concentration of your sample from the assay, make sure to take into account the dilution factor.
5. Avoid cross-contamination by using new applicator and suction tips. Also use a new membrane to seal the microplate during the assay.

Specimen requirements

1. Avoid preserving specimen in sodium azide, which inhibits HRP in the assay.
2. Store specimen at -80 ^{o}C, avoid freeze-thawing cycles.

Assay procedure

1. Dilute Standard (Table 1, item 1) in Standard Diluent (Table 1, item 2) in a 1.5 ml tube to make reference standard solutions as shown in Table 2. Configure duplicate samples for each dilution.

Table 2. Standard dilutions.

480ng/L	Standard No.5	120µl Standard + 120µl Standard diluent
240ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
120ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
60ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
30ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

2. Dilute 30X Wash Solution in dH_20 (Table 1, item 5). Make 3 ml of diluted wash solution for each assay well.
3. On the assay strip plate, designate 10 wells for standards (each standard in duplicate) and one well for the blank. Add 50 µl diluted standards to each of the 10 standard wells. In the "blank" wells, add 50 ml Sample Diluent (Table 1, item 6). For experimental samples, add 40 □l Sample Diluent (Table 1, item 6) and 10 µl sample in each sample well; therefore, the sample dilution is 5X. If the concentration of sample is high and saturates the assay, you may have to perform further dilution of the sample, as determined empirically. Seal the corners of the microplate with parafilm and mix for 1 hr at 37 ^{o}C, using a rocker table.
4. Discard liquid in wells by gently aspirating. Turn the plate upside down and pat dry the plate with an absorbent paper. To wash the wells, fill each well with 100 ml of diluted wash solution (Table 1, item 5), oscillate with the rocker table for 30 sec, and then aspirate off liquid. Pat dry the microplate with absorbent paper. Repeat the wash steps 5 times.
5. Add 50 ml HRP-conjugate reagent (Table 1, item 4) to each well, except for the blank well. Shake gently for 1 hr at 37 ^{o}C.
6. Wash the wells as described in step 4.
7. Add 50 µl of Chromogen Solution A (Table 1, item 7) and 50µl of Chromogen Solution B (Table 1, item 8), gently mix for 10 min at 37 ^{o}C.
8. Add 50 µl of Stop Solution (Table 1, item 9) to each well. Upon addition of stop solution, the blue color should change to yellow immediately.
9. Measure the optimal density (OD) at 450 nm within 15 minutes of adding the stop solution. Set the blank well as zero.

Data Analysis

10. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to calculate the concentration of your samples. Remember that samples were diluted 5 fold in the assay in your final calculation. Other forms of analysis to calculate concentrations of your samples can also be

performed. The assay sensitivity is between 15ng/L to 500 ng/L.

Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at 37 ℃

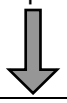
Wash each well five times.

Add HRP conjugate to each well, incubate for 1 hr at 37 ^{o}C

Wash each well five times

Add Chromogen Solution A and B, 10 min at 37 ^{o}C

Add stop solution



Measure OD 450 nm within 15 min