NovaTeinBio

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****** Competition ELISA Kit Instruction

Kit name and catalog number

Your analyte ELISA Kit, Catalog#: *****

Intended use

The kit is used to detect the level of Your analyte in cell culture, serum, blood plasma and other suitable sample solution.

Assay principle

The coated well immunoenzymatic assay for the quantitative measurement of analyte utilizes a multiclonal anti-analyte antibody and an analyte-HRP conjugate. The assay sample and buffer are incubated together with analyte-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 analyte concentration since analyte from samples and analyte -HRP conjugate compete for the anti-analyte antibody binding site. Since the number of sites is limited, as more sites are occupied by analyte from the sample, fewer sites are left to bind analyte-HRP conjugate. Standards of known analyte concentrations are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density) to the concentration of analyte. The analyte concentration in each sample is interpolated from this standard curve.

Manufactured and distributed by:

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Materials supplied

1	Microelisa Stripplate	96 well	6	Chromogenic Substrate A	6ml X 1vial
2	Standard	1.0 ml X 6 vials	7	Chromogenic Substrate B	6ml X 1 vial
3	100 X Wash Solution	10ml X 1vial	8	Stop Solution	6ml X 1 vial
4	Lysis Buffer Solution	6ml X 1vial	9	Specification	1
5	HRP-Conjugate Reagent	6ml X 1 vial			

Note: Standard (S1 → S6) concentration was followed by: Variable with different kit

Sample collection and storage

Serum-Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the 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 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids-Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

NOTE: The Lysis Buffer Solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; if the sample is serum or blood plasma, then the Lysis Buffer Solution is a superfluous reagent. Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤2months) or -80°C(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Materials required but not supplied

1. 37℃ incubator
2. Standard plate reader capable of measuring absorbance at 450 nm.
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Multi-channel pipettes, manifold dispenser or automated microplate washer.
6. Absorbent paper

Sample Preparation

1. Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25 ℃) before use.
2. Dispense 10 µl of Lysis Buffer Solution into 100µl specimens, mix and stand for one hour (The proportion of Lysis Buffer and Specimens should be no less than 1:10). (NOTE: This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; if the sample is serum or blood plasma, then this step should be skipped.)
3. Wash Solution - Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

Assay procedures

Prepare all Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.
1. Secure the desired numbers of coated wells in the holder then add 100µl of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Generally, you should get the sample value within the assay arrange without dilution. If samples generate values higher than the highest standard, further dilute the samples with 0.1%BSA in PBS, pH7.4 (without Mg^{2+}, Ca^{2+}) and repeat the assay.
2. Add 50µl of Conjugate to each well. Mix well. Mixing well in this step is important. Cover and incubate the plate for 1 hour at 37°C.
3. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl /well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

4. Add 50µl Chromogenic Substrate A and 50µl Chromogenic Substrate B to each well, subsequently. Cover and incubate for 10 minutes at 20-25°C. (Avoid sunlight).

5. Add 50µl of Stop Solution to each well. Mix well.

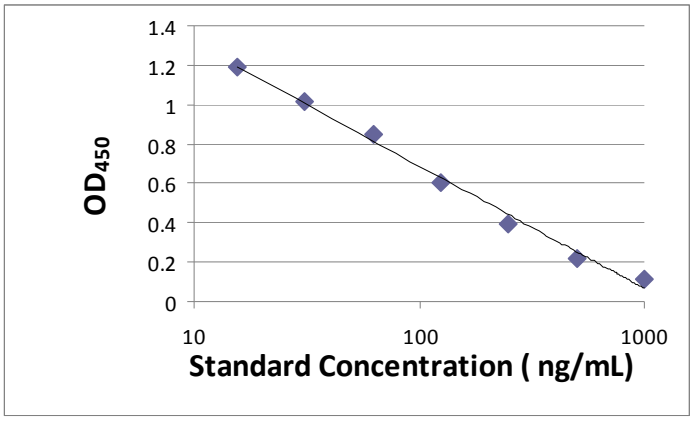
6. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Important notes

1. The operation should be carried out in strict accordance with the provided instructions.
2. To preserve unused strip-wells, it should be stored in the sealed bag.
3. Always avoid foaming when mixing or reconstituting protein solutions.
4. Pipette reagents and samples into the center of each well.
5. The samples should be transferred into the assay wells within 15 minutes of dilution.
6. We recommended that all standard, testing samples are tested in duplicate to minimize the test errors.
7. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Avoid cross-contamination by changing tips, using separate reservoirs for each reagent, avoid using the suction head without extensive wash.
9. Do not mix the reagents from different batches
10. Stop Solution should be added in the same order of the Substrate solution.
11. Chromogenic Substrate B is light-sensitive, please avoid prolonged exposure to light.
12. The kit should be kept at 2 - 8 ℃ and cannot be used after expiration date. Standards to be used within 5 days may be stored at 2-8℃ , otherwise Standards must be stored at -20℃ to avoid loss of bioactivity .

Result calculation

1. This standard curve is used to determine the amount of an unknown sample. Construct a standard curve by plotting the average O.D. (450 nm) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. Standard curve (reference in general, not for this kit in particular):



Sensitivity and specificity

1. The sensitivity in this assay is 0.1ng/ml (Variable).
2. This assay has high sensitivity and excellent specificity for detection of analyte. No significant cross-reactivity or interference between analyte and analogues was observed.
3. Storage: 2-8℃.
4. Validity: six months.