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User's Manual

Bovine ITIH4 ELISA Kit

REF **DEIABL386**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This Bovine ITIH4 ELISA Kit allows for the in vitro quantitative determination of target antigen concentrations in serum, plasma, tissue homogenates, cell culture supernatants or other biological fluids.

Principles of Testing

The ELISA is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to target antigen, During the reaction, target antigen in the sample or standard competes with a fixed amount of biotin-labeled target antigen for sites on a pre-coated antibody specific to target antigen. Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of target antigen in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

1. ELISA Microplate: Stored at -20°C
2. Standard: 2 vial. Stored at -20°C
3. Sample Diluent: 1 × 20 mL. Stored at -20°C
4. Assay Diluent A: 1 × 10 mL. Stored at -20°C
5. Assay Diluent B: 1 × 10 mL. Stored at -20°C
6. Detection Reagent A: 1 × 60 µL. Stored at -20°C
7. Detection Reagent B: 1 × 120 µL. Stored at -20°C
8. Wash Buffe (25× concentrate): 1 × 30 mL. Stored at 4°C
9. Substrate: 1 × 10 mL. Stored at 4°C
10. Stop Solution: 1 × 10 mL. Stored at 4°C
11. Plate sealer: 5

Storage

2-8°C/-20°C

Specimen Collection And Preparation

These are general guidelines for preparing common samples in ELISA assays.

1. **Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 ×g. Remove serum and assay immediately or aliquot and store

samples at -20°C or -80°C.

2. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g at 2°C - 8°C within 30 minutes of collection. Store samples at -20°C or -80°C.
3. **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with ice-cold 1× PBS to remove excess blood, homogenized in ice-cold 1× PBS and stored overnight at ≤ -20°C. In most cases, 10% homogenate (eg. 1 g of tissue in 10 mL of ice - cold 1× PBS) is recommended.After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 ×g. Remove the supernatant and assay immediately or aliquot and store at ≤ -20°C.
4. **Cell culture supernatants and Other biological fluids:** Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C.

Fresh samples are the first choice. If not, avoid freeze-thaw of samples.

Reagent Preparation

1. **Standard**: Please refer to the Data Sheet inserting in the kit.
2. **Detection Reagent A and B:** Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.
3. **Wash Buffer:** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Please accurately prepare the working solutions on demand before use.

Assay Procedure

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C directly). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at -20°C unti the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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.

1. Add 50 µL of **Standard**, **Blank**, or **Sample** per well.
2. Immediately add 50 µL of **Detection A** working solution to each well. Cover with the **Plate sealer**. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with **Wash Buffer** (approximately 300 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1 - 2 minutes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection Reagent B** working solution to each well. Cover with a new **Plate sealer**. Incubate for 45 minutes at 37°C.
5. Repeat the aspiration/wash process for five times as conducted in step 3.

6. Add 90 µL of **Substrate Solution** to each well. Cover with a new **Plate sealer**. Incubate within 10 - 20 minutes at 37°C. Protect from light.
7. Add 50 µL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Calculation

Average the duplicata readings for each standard, control, and sample. Create a standard curve by mean optical density using computer software capable of generating a four parameter logistic (4 - PL) curve - fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x - axis against the concentration on the y - axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentrations versus the log of the O.D, and the best fit line can be determined by regression analysis. It is recommended to use some professional software to do this calculation, such as Curve Expert. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Detection Range

0.312 - 20 ng/mL

Detection Limit

0.087 ng/mL

Specificity

Natural and recombinant bovine Inter-alpha-trypsin inhibitor heavy chain H4

Precautions

1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted **Standards, Detection Reagent A and B**can be used only once.
2. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.
3. To avoid cross - contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. **Substrate Solution** is easily contaminated. Please protect it from light.

6. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to printed instruction inside in the kit while electronic one from our website is for reference only.
7. Do not substitute reagents from one lot to another. Use only the reagents in the same kit supplied by manufacturer.
8. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
9. Each kit has strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers.
11. Period of validity: six months.
12. The **Stop Solution** provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.