Sheep arachidonic Acid (AA) ELISA kit

Catalog Number: orb339635

- This immunoassay kit allows for the in vitro quantitative determination of **sheep AA** concentrations in **serum, plasma, tissue homogenates**.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to AA has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with HRP-conjugated AA and incubated. A competitive inhibition reaction is launched between AA (Standards or samples) and HRP-conjugated AA with the pre-coated antibody specific for AA. The more amount of AA in samples, the less antibody bound by HRP-conjugated AA. Then the substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of AA in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

62.5 ng/ml-1000 ng/ml. The standard curve concentrations used for the ELISA's were 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 62.5 ng/ml.

SPECIFICITY

This assay recognizes sheep AA. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of sheep AA is typically less than 15.6 ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	2 x 20 ml
HRP- conjugate Diluent	1 x 10 ml
HRP- conjugate	1 x 60 µl
Wash Buffer	1 x 20 ml (25×concentrate)
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

STORAGE

- Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.

- Opened test plate should be stored at 2-8°C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

- A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.

1. **HRP- conjugate** Centrifuge the vial before opening. Dilute to the working concentration using **HRP-conjugate Diluent** (1:100), respectively.

2. **Standard**

- Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. This reconstitution produces a stock solution of 1000 ng/ml. The 1000ng/ml standard serves as the high standard(tube#1). Label five tubes#2 through #6.

- Pipette 150µl Sample Diluent into tubes#2-6. Add 150µl of the 1000 ng/ml standard（**tube #1**） to tube #2. Vortex thoroughly. Add 150µl of tube #2 to tube #3 and vortex thoroughly,Continue this for tubes #3 through #5. Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 ng/ml) **(tube #6)**.

The concentration of sheep AA in tubes #1through #6 will be 1000, 500, 250, 125, 62.5 and 0 ng/ml respectively.

Diluted standards should be used within 30 minutes of preparation.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

- Pipettes and pipette tips.

- Deionized or distilled water.

- Squirt bottle, manifold dispenser, or automated microplate washer.

- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

- **Serum -** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Tissue Homogenates 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARTION

Recommend to dilute the serum or plasma samples with Sample Diluent(1:500) before test. The suggested 500-fold dilution can be achieved by adding 5µl sample to 95µl of Sample Diluent first, then complete the 500-fold dilution by adding 10µl of this solution to 240µl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Set a Blank well without any solution.

2. Add 50µl of Standard or Sample per well. Add 50µl **HRP-conjugate working solution** to each well immediately. Mix well with the pipette or shake the plate gently for 60 seconds.

3. Then incubate for 40 minutes at 37°C.

4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash: Fill each well with Wash Buffer (200µl) and let it stand for 2 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.

5. Add 90µl of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

6. Add 50µl of **Stop Solution** to each well when the last four wells containing the lowest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, and sample and subtract the average optical density of Blank. Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the absorbance ratio 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 AA concentrations versus the ratio and the best fit line can be determined by regression analysis. 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.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
  If samples generate values higher than the highest standard, dilute the samples with the appropriate Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
  To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless or light blue to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.