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User's Manual

Swine Interleukin 2, IL2 ELISA Kit

REF **CKERS-IL2-193S**

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RUO

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This assay is a sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). It is developed for quantitative measurement of Swine IL2 in serum, plasma and other biological fluids.

Principles of Testing

An antibody specific for Swine IL2 is coated onto the wells of the microtiter plate. Samples and standards of Swine IL2 are pipetted into the wells for binding to the coated antibody. After washing procedure to remove unbound compounds, an enzyme-linked antibody specific for Swine IL2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Swine IL2 bound in the initial step. The color development is stopped and the intensity of the color is measured. The magnitude of the absorbance for this developed color is proportional to the amount of Swine IL2.

Reagents And Materials Provided

Swine IL2 Microplate: polystyrene microplate coated with a monoclonal antibody against Swine IL2;

Standard (freeze dried): 500 pg/mL, 2 vials;

Standard or Sample Diluent: 16ml, 1 vials;

Biotin-antibody (100×): 60ul, 2 vials;

Biotin-antibody Diluent: 16ml, 1 vials;

HRP-avidin (100×): 60ul, 2 vials;

HRP-avidin Diluent: 16ml, 1 vials;

TMB Substrate: 12ml, 1 vials;

TMB Stop Solution: 12ml, 1 vials;

Wash Buffer (20×): 25ml, 1 vials;

Microtiter plate sealers

Storage

Unopened Kit: Store at 2-8°C. Do not use past kit expiration date.

Reagent Preparation

Sample: Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1, 000×g. Remove particulates and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Standard: Centrifuge the standard vial at 6, 000-10, 000rpm for 30s. Reconstitute the Standard with 1.0 ml of standard or Sample Diluent. The undiluted standard serves as the high standard (500 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL). Prepare fresh for each assay. Prepare within 2 hours of use.

Biotin-antibody: Centrifuge the vial before opening. Dilute to the working concentration using Biotin-antibody Diluent(1:100), respectively. Prepare within 1 hour of use.

HRP-avidin: Centrifuge the vial before opening. Dilute to the working concentration using HRP-avidin Diluent(1:100), respectively. Prepare within 1 hour of 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× Wash Buffer Concentrate into deionized or distilled water.

Assay Procedure

1. Prepare all reagents, working standards, and samples as directed in the previous sections. Dilute original density Standard as follow: Set up 7 points of diluted standard such as 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.625 pg/mL and 7.8 pg/mL. The last EP tubes with Sample Diluent is the blank as 0 pg/mL.
2. Add 100µlof Standard, Control or Sample per well. Cover with the Microtiter plate sealers. Incubate for 1.5 hours at 37°C.
3. Aspirate each well and wash, Wash by filling each well with 1 x Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Repeating the process twice for a total of four washes. After the last wash, remove any remaining 1× Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100µlof Biotin-antibody working solution to each well. Cover with the Microtiter plate sealers. Incubate for 1 hour at 37°C. Biotin-antibody working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
5. Repeat the Aspirate/Wash four times.
6. Add 100µlof HRP-avidin working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 0.5 hours at 37°C.
7. Repeat the Aspirate/Wash four times.
8. Add 100ul TMB Substrate to each well. Mix gently, **protected from light** and incubates at 37°C for 10-20 min.
9. Add one drop (100µl) of TMB Stop Solution to each well to stop the color reaction. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450nm.

Calculation

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the sample concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding sample concentration. If samples have been

diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Detection Range

7.8-500 pg/mL

Detection Limit

7 pg/mL

Precautions

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. 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.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.