Version 28-02/25

User's Manual

Pig calprotectin (CALP) ELISA Kit

REF DAEF-012

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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 kit is designed for the quantitative determination of pig calprotectin(CALP) concentrations in serum, plasma, tissue homogenates.

Principles of Testing

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with CALP. Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated antibody preparation specific for CALP. The competitive inhibition reaction is launched between with pre-coated CALP and CALP in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of CALP in the samples. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Assay plate-1(96 wells)
2. Standard (Freeze dried)-2
3. HRP-conjugate (100 x concentrate)-1 x 60 µl
4. HRP-conjugate Diluent-1 x 10 ml
5. Sample Diluent-2 x 20 ml
6. Wash Buffer (25 x concentrate)-1 x 20 ml
7. TMB Substrate-1 x 10 ml
8. Stop Solution-1 x 10 ml
9. Adhesive Strip (For 96 wells)-4
10. Instruction manual-1

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100ml and 500ml graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

Storage

Store at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Serum Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g, 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

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 removed and assayed 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.

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.

Reagent Preparation

1. HRP-conjugate (1x) - Centrifuge the vial before opening.

HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP-conjugate Diluent.

2. Wash Buffer(1x)- 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 (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

3. Standard

Centrifuge the standard vial at 6000-10000rpm for 30s.

Reconstitute the Standard with 1.0 ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 20 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 150 µl of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (20 ng/ml). Sample Diluent serves as the zero standard (0ng/ml).

Assay Procedure

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 50µl of standard and sample per well. Then add 50µl of HRP-conjugate (1x) to each well(Not to Blank well). Cover the microtiter plate with adhesive strip. Incubate for 30 minutes at 37°C. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to 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.
6. Add 90µl of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light.
7. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation

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 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 CALP concentrations versus the log of the O.D. 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.

Precision

Intra-assay Precision: CV%<8%

Inter-assay Precision: CV%<10%

Detection Range

0.312 ng/ml-20 ng/ml.

Sensitivity

The minimum detectable dose of pig CALP is typically less than 0.078 ng/ml.