Porcine Immunoglobulin M(IgM) ELISA Kit

Catalog No. CSB-E06805p

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **porcine IgM** concentrations in **serum, plasma.**

- **Expiration date**   **six** months from the date of manufacture

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with porcine IgM. Standards or samples are then added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) -conjugated antibody preparation specific for porcine IgM，mix well and incubated. The more the amount of porcine IgM in samples, the less HRP-conjugated antibody preparation specific for porcine IgM bound by pre-coated porcine IgM. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. And the color develops in opposite to the amount of porcine IgM in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.04µg/ml-10 µg/ml. The standard curve concentrations used for the ELISA's were 10 µg/ml, 2.5µg/ml, 0.62 µg/ml, 0.16 µg/ml, 0.04 µg/ml.

SPECIFICITY

This assay recognizes porcine IgM. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of porcine IgM is typically less than 0.04µg/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	6×0.5ml				
Sample Diluent	2 x 20 ml				
HRP -conjugate	1 x 6 ml				
Wash Buffer	1 x 20 ml (25×concentrate)				
TMB Substrate	1 x 10 ml				
Stop Solution	1 x 10 ml				
Standard	S0	S 1	S2	S3	S4	S5
Concentration (µg/ml)	0	0.04	0.16	0.62	2.5	10

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. 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.

1. **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.

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.

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. 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.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARTION

Recommend to dilute the serum or plasma samples with Sample Diluent(1:2000) before test. The suggested 2000-fold dilution can be achieved by adding 5µl sample to 195µl of Sample Diluent. Complete the 2000-fold dilution by adding 5µl of this solution to 245µl 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.

1. Set a **Blank** well without any solution.
2. Add 50µl Standard or Sample to per well. Add 50ul HRP-conjugate 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, Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Repeating the process for a total of five time washes. 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.
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

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 porcine IgM 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.
- Any variation in Standard Diluent, 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 until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless 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.