NOVATEINBIO
Accelerates Research and Development

Porcine Cytomegalovirus (CMV) IgG Antibody ELISA Kit

Catalog number: BG-POR10453 (96 wells)

The kit is designed to qualitatively detect CMV IgG in Porcine serum.

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that the positive control, negative control and test samples are tested in duplicate.
- If the blue color develops too shallow after 10 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- The TMB reagent is light-sensitive. Avoid prolonged exposure to the light.

Intended use

This kit is used to to qualitatively detect CMV IgG in porcine serum.

Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

This assay employs the enzyme-linked immunosorbent assay (ELISA) technology to detect CMV IgG in the samples. Purified CMV antigen was precoated in microplate wells. The CMV IgG in the sample, if presents, will bind to the antigen immobilized on the wells and interacts with anti-porcine IgG conjugated with HRP to form an immunocomplex. Following incubation and washing procedures to remove unbound substances, this reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). After stopping the reaction with sulfuric acid, the blue color turns yellow. What can be measured at this point is the amount of color intensity proportional to the amount of antibody captured in the wells, and to the sample.

Materials supplied

1	Microplate precoated with CMV antigen	1x96 well
2	Negative Control	1 vial
3	Positive Control	1 vial
4	Calibrator	1 vial
5	Diluent buffer	30 ml
6	Wash Solution (10x)	100 ml
7	Anti- porcine IgG -HRP Conjugate	12 ml
8	TMB developing reagent	12 ml
9	Stop Solution	12 ml
10	Closure plate membrane	2
11	Sealed bag	1
12	Package insert	1

Materials required but not supplied

- 37°C incubator.
- Standard microplate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 3000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.

NOTE: Serum to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 2 months) or -80°C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Sample Preparation

- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- Wash Solution - Dilute 100 mL of Wash Solution concentrate (10×) with 900 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

Assay procedures

1. Prepare all the controls and samples before starting assay procedure (Please read Reagents Preparation).
2. Set one blank control well (no serum should be added into the Blank well), two calibrator wells, two Negative control wells, two Positive control wells and test sample wells on the assay plate.
3. Dilute test samples, calibrator and controls 1:40 in Diluent buffer. Add 100 µl of diluted calibrator, Positive control, Negative control, and samples into their respective wells. Add 100 µl of Diluent buffer to the Blank well. Mix well. Cover and incubate the plate for 30 minutes at room temperature.
4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

   Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400µl/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
5. Add 100 µl of HRP conjugate into each well, mix well. Cover and incubate the plate for 30 minutes at room temperature.
6. Discard the solution and wash the plate as step 4.

7. Add 100 µl TMB developing reagent to each well, subsequently. Cover and incubate for 10-15 minutes at room temperature (Protect from light. Do not over develop).
8. Add 100 µl Stop Solution to each well, following the same order of TMB developing reagent addition. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader ***immediately***.

Determine the Results

Calculations of results

1. Calculate the mean of duplicate calibrator value

2. Calculate the mean of duplicate positive control, negative control and samples.

3. Calculate CMV IgG index of each determination: Divide the mean values of each sample by calibrator mean value

Quality control

For the assay to be considered valid, the following conditions must be met:

1. Calibrator and Controls must be run with each test run.

2. The OD value of Reagent blank (when read against air blank) must be < 0.250 at 450 nm.

3. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.

4. The CMV IgG Index for Negative and Positive Control should be in the range stated on the labels.

Interpretations of the results

- Positive Result: If the CMV IgG Index of sample is greater than, or equal to 1.0, the sample is considered to be seropositive for IgG antibody to CMV
- Equivocal Result: If the CMV IgG Index of sample is between 0.91 - 0.99, the result is considered to be equivocal. Sample should be retested
- Negative Result: If the CMV IgG Index of sample is less than 0.90, the sample is considered to be seronegative for IgG antibody to CMV

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