Mouse Parvovirus ELISA Kit

Cat #: orb1821043 (manual)

Product Description

Mouse Parvovirus ELISA kit is for the qualitative determination of Parvovirus (PV) in Mouse serum, plasma, culture media or any biological fluid.

Components

Component Name	Storage Conditions	96 reactions
Plate Cover	R.T.	2
Self-sealing Bag	R.T.	1
ELISA strip Microplate	2-8°C	1
Negative Control	2-8°C	0.5ml×1 bottle
Positive Control	2-8°C	0.5ml×1 bottle
HRP-Conjugate Reagent	2-8°C	6ml×1 bottle
Sample Diluent	2-8°C	6ml×1 bottle
Chromogen Solution A	2-8°C	6ml×1 bottle
Chromogen Solution B	2-8°C	6ml×1 bottle
Stop Solution	2-8°C	6ml×1 bottle
Wash solution (30X)	2-8°C	20ml×1bottle

Storage and Stability

Store all reagents at 2-8°C. Product stored under these conditions should be stable for 6 months.

Scientific Background

The microplate provided in this kit has been pre-coated with an antibody specific to PV. Samples are added to the microplate wells and bind the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for PV is added to each well and incubated forming an antibody-antigen-enzyme labeled antibody complex. Following a wash to remove any unbound reagent, TMB substrate solution is added to each well. Wells that contain PV and HRP conjugated PV antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of PV is determined by comparing with the CUTOFF value.

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Sample Preparation

Notes:

- Sample extraction and ELISA assay should be performed as soon as possible after sample collection. Samples should be extracted according to the relevant literature. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Repeated freeze-thaw cycles should be avoided.
- These kits cannot be used for samples with NaN3 which can inhibit the activity of HRP.

Serum Samples

Collect whole blood. Allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during storage, the sample should be centrifuged again.

Plasma Samples

Collect whole blood into tubes with anticoagulant (EDTA or citrate). After incubation at room temperature for 10-20 minutes, centrifuge tubes for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during storage, the sample should be centrifuged again.

Urine, Cerebrospinal fluid, and Pleuroperitoneal Samples Collect urine in aseptic tubes. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. If precipitates appear during storage, the sample should be centrifuged again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine samples.

Cell Samples

To detect cell secretions, collect culture supernatant into aseptic tubes. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. To detect intracellular components, dilute the cells to 1X106/ml with PBS (pH 7.2- 7.4). Destroy the cells by repeated freezing and thawing to release intracellular components. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. If precipitates appear during storage, the sample should be centrifuged again.

Tissue samples

Cut, weigh, and freeze tissue samples in liquid nitrogen. Store at -80°C for future use. Thaw at 4°C prior to use. Homogenize samples after adding PBS (pH 7.4). Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Procedure

Notes:

- The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from the antibody-coated ELISA strip microplate, reseal them in self-sealing bag and keep at 4°C.
- Precipitates may appear in the concentrated wash solution. Please heat the solution to dissolve all the precipitates, this will not affect the results.
- To avoid cross-contamination, plate covers are for one- time use only.
- Keep Chromogen Solutions A and B away from light.
- All absorbance reading operations should be conducted strictly in accordance with the Microplate Reader manufacturer's instructions.
- All samples and waste products should be treated as infectious agents.
- Reagents from different lots should not be mixed.

Step 1: Number the microplate wells as appropriate for each sample, leave two wells as negative control, two wells as positive control, and one empty well as blank control. In the blank control well, sample and HRP- conjugate reagent will not be added, the rest of the steps will be the same.

Step 2: Add 50µl of negative and positive control to the negative and positive control wells respectively. In the sample wells, add 40µl of sample diluent and 10µl sample (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well by gently shaking.

Step 3: Seal plate with plate cover and incubate for 30 min at 37°C.

Step 4: Dilute the 30X wash solution with distilled water to a final concentration of 1X. The entire bottle may be diluted and stored at 2-8°C. Otherwise, note that approximately 5ml of 1X wash solution will be needed for each well.

Step 5: Carefully remove plate cover, aspirate well contents, and refill with 450 – 500µl the wash solution. Discard the wash solution after resting for 30 seconds. Repeat this washing procedure a total of 5 times.

Step 6: Add 50µl HRP-Conjugate reagent to each well except the blank control well. Incubate as described in Step 3, then wash as described in Step 5.

Step 7: Add 50µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix by gently shaking and incubate at 37 ℃ for 15 minutes. Avoid exposure to light during this step.

Step 8: Add 50µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

Step 9: Read absorbance O.D. at 450nm using a Microplate Reader. The OD value of the blank control well should be set to zero. The absorbance should be read within 15 minutes of adding the stop solution.

Determine the result.

Test effectiveness:

average value of positive control ≥1.00

average value of negative control ≤0.10

Critical value (CUT OFF) calculation:

critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Mouse PV negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Mouse PV positive.

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