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IMMUNOPORT

SKU IP201165

ELISA KIT FOR RAT TMEV

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.2B

INTENDED USE

The kit is a two-site sandwich enzyme immunoassay for in vitro quantitative measurement of Rat Theiler's Murine Encephalomyelitis Virus (TMEV) in serum, plasma, tissue homogenates, cell culture supernates and other biological fluids.

KIT CONTENTS & STORAGE

Rat TMEV Microplate [12 X 8 Strips]
Rat TMEV Standard [0.5mL]
HRP-Conjugated Rat TMEV Detection Antibody [6mL]
Standard Diluent [1.5mL]
Sample Diluent [6mL]
Chromogen Solution A and B [6mL each]
Stop Solution [6mL]
Wash Buffer [20mL (30x)]
Plate Sealing Membrane [2pcs]
User manual [1pc]

Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8°C.

NOT INCLUDED

ELISA Reader, 37°C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

PRINCIPLE AND SUMMARY

Rat Theiler's Murine Encephalomyelitis Virus (TMEV) ELISA Kit employs a two-site sandwich ELISA to quantitate TMEV in samples. An antibody specific for Rat TMEV has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TMEV present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated TMEV detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of TMEV bound in the initial step. The color development is stopped and the intensity of the color is measured.

CHARACTERISTICS

Sample Type: Serum, plasma, tissue homogenates, cell culture supernates and other biological fluids.

Detection Range: 5 pg/mL - 80 pg/mL
Minimum Detection Dose: 0.5 pg/mL
Intra-Assay Precision: CV<9%
Inter Assay Precision: CV<11%
Linearity with spiked analytes: ρ=0.99
Recovery Range: 98-116% with mean of 106% with spiked samples
Cross Reactivity: None observed with analogues.
Validity: 1 year from the date on manufacture
Storage: +2-8°C
Once opened unused wells should be put into the sealed bag with a desiccant pack and may be used for up to 1 month.

SAMPLE COLLECTION

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

Cell Culture Supernatants and Other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.
4. Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

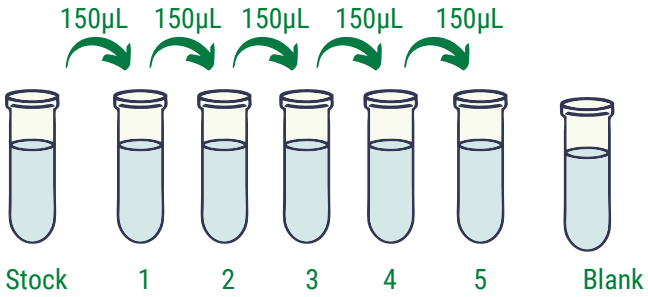
REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Note: The dilution of Wash Buffer and Sample Diluent/ Assay Buffer in the following operation steps is based on 96T kit. If a 48T kit is used, please scale it down.

Wash Buffer - Dilute with Distilled or deionized water 1:20 (48T) /1:30 (96T)

Standard - Pipette 150 µL of Standard Diluent into each tube. 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.



Concentrations
Stock- 160pg/mL
1- 80pg/mL
2- 40pg/mL
3- 20pg/mL
4- 10pg/mL
5- 5pg/mL
Blank- 0pg/mL

Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 µL to standard well.

3. Add Sample: Add sample diluent 40 µL to testing sample well. Then add sample 10 µL to testing sample well, Blank well doesn't add anything.

4. Cover with a plate cover and incubate for 45 minutes at 37 °C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, manifold dispenser or autowasher. 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 HRP-Conjugated detection antibody 50 µL to each well, except blank well.

7. Cover with plate cover. Incubate for 30 minutes at 37 °C.

8. Repeat the aspiration/wash process for five times as in step 5.

9. Add chromogen solution A 50 µL and chromogen solution B 50 µL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light. The reaction should change from colorless to gradations of blue.

10. Add 50 µL Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). 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 for each standard 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 Rat TMEV 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.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at customerservice@lifetechindia.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources. 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.

- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

- Substrate solution should remain colorless until added to the plate. Keep chromogen solution protected from light.

- Stop Solution should be added to the plate in the same order as the chromogen 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.

- Stop Solution contains 2N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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