Version 24-04/24

User's Manual

Mouse Hepatitis Virus(HV) Elisa Kit

REF **DEIA7161**

Σ **96T**

RUO

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This Kit allows for the determination of MHV concentrations in Mouse serum, cellculture supernates and other biological fluids.

General Description

Viral hepatitis is liver inflammation due to a viral infection. It may present in acute form as a recent infection with relatively rapid onset, or in chronic form. The most common causes of viral hepatitis are the five unrelated hepatotropic viruses hepatitis A, B, C, D, and E. Other viruses can also cause liver inflammation, including cytomegalovirus, Epstein-Barr virus, and yellow fever.

Principles of Testing

Mouse Hepatitis Virus (MHV) ELISA Kit employs a two-site sandwich ELISA to quantitate MHV in samples. An antibody specific for Mouse MHV has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MHV present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated MHV 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 MHV bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Mouse MHV microplate: 96 wells
2. Mouse MHV standard: 0.5 mL
3. HRP-Conjugated Mouse MHV detection antibody: 6 mL
4. Standard diluent: 1.5 mL
5. Sample diluent: 6 mL
6. Chromogen solution A: 6 mL
7. Chromogen solution B: 6 mL
8. Stop solution: 6 mL
9. Wash buffer: 20 mL (30×)
10. Plate covers: 2pcs
11. Booklet: 1pcs

Materials Required But Not Supplied

1. 37 °C incubator.
2. Standard microplate reader capable of measuring absorbance at 450 nm.

3. Precision pipettes, disposable pipette tips and Absorbent paper.
4. Distilled or deionized water.

Storage

Store kit reagents at 2-8 °C for 6 months. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Specimen Collection And Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should 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 which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

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 approximately 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 1000×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.

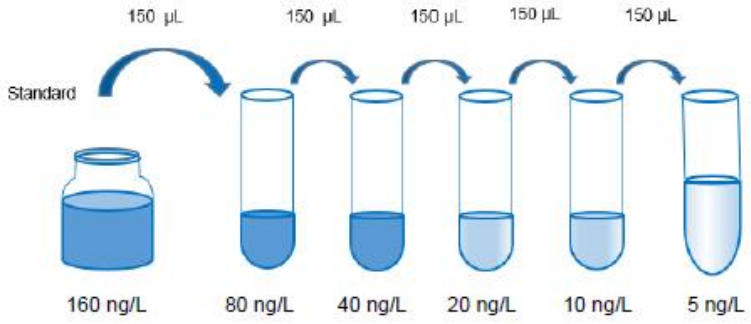
Note: Samples should be centrifugated adequately and no hemolysis or granule was allowed.

Reagent Preparation

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

Wash buffer - Dilute with Distilled or deionized water 1:30.

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. Standard Diluent serves as the zero standard.



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

Assay Procedure

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 tostandard 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 fivewashes, 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 ateach step is essential to good performance. After the last wash, remove any remainingWash buffer by aspirating or decanting. Invert the plate and blot it against clean papertowels.
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. Gentlymix and incubate for 15 minutes at 37°C. Protect from light.
10. Add 50 µL Stop Solution to each well. The color in the wells should change from blueto 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 15minutes.

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 Mouse SeV 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.

Typical Standard Curve

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Detection Range

5 ng/L - 80 ng/L

Detection Limit

The minimum detectable dose (MDD) of Mouse MHV is typically less than 0.5 ng/L.

Specificity

Mouse Hepatitis Virus (MHV) ELISA Kit has high sensitivity and excellent specificity fordetection of Mouse MHV. No significant cross-reactivity or interference betweenMouse MHV and analogues was observed.

Linearity

To assess linearity of the assay, samples containing and/or spiked with highconcentrations of Mouse MHV were diluted with the appropriate calibrator diluent toproduce samples with values within the dynamic range of the assay. Linear regressionanalysis of samples versus the expected concentration yielded a correlation coefficientof 0.99.

Recovery

Four samples of known concentration were tested twenty times on one plate to assessintra-assay precision. The CV (%)<9%.

Three samples of known concentration were tested in twenty separate assays toassess inter-assay precision. Assays were performed by at least three techniciansusing two lots of components. The CV (%)<11%.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biologicalsamples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding.

4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standardlevel, between sample additions, and between reagent additions. Also, use separatereservoirs for each reagent.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps isnecessary.
7. When using an automated plate washer, adding a 30 second soak period following theaddition of Wash Buffer, and/or rotating the plate 180 degrees between wash stepsmay improve assay precision.
8. Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
9. Stop Solution should be added to the plate in the same order as the Chromogensolution. The color developed in the wells will turn from blue to yellow upon addition ofthe Stop Solution. Wells that are green in color indicate that the Stop Solution has notmixed thoroughly with the Chromogen solution.
10. 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 fromRat blood will not transmit infectious agents. Therefore, all blood derivatives should beconsidered potentially infectious and good laboratory practices should be followed.
11. All samples should be disposed of in a manner that will inactivate viruses.
12. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The wasteshould be allowed to stand for a minimum of 30 minutes to inactivate the viruses beforedisposal.