Epizootic Diarrhea of Infant Mice, Mouse Rotavirus (Mouse Sera)

Catalog # SMART-M19
For the detection of antibodies to Epizootic Diarrhea of Infant Mice (EDIM) in Mouse Sera by ELISA
For Use On Research Animals Only

ASSAY PRINCIPLE
The micro test wells are coated in alternating columns with Epizootic Diarrhea of Infant Mice (EDIM) Antigen and Control-Antigen. During the first incubation with the diluted specimen, any antibodies that are reactive with the EDIM Antigen or Control-Antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction is then read with an ELISA reader and an Index Value is calculated from the Differential OD (Antigen well OD minus Control-Antigen well OD) of the specimen.

REAGENTS PROVIDED
Test Strips: Two 96-breakaway well Microplates in holders coated in alternating columns with EDIM Antigen and Control-Antigen. The Antigen wells are ringed in black and the Control-Antigen wells are ringed in red.
Enzyme Conjugate: One 11ml bottle of anti-mouse IgG conjugated to peroxidase
Positive Control Serum: One 1ml vial of diluted positive serum
Negative Control Serum: One 1ml vial of diluted negative serum
Calibrator: One 100μl vial of positive serum
Chromogen: One 11ml bottle of TMB
Wash Buffer: Three 25ml bottles of concentrated (20X) buffer with surfactant
Dilution Buffer: Two 30ml bottles of buffered protein solution
Stop Solution: One 11ml bottle of 1M Phosphoric Acid

REAGENT STORAGE
Store the reagents, strips and bottled components between 2 - 8º C.
The diluted wash buffer may be stored at room temperature for up to 180 days, but do not use if it becomes cloudy. Do not add fresh buffer to old buffer.

SERUM COLLECTION AND HANDLING
This test utilizes the specimen’s serum: coagulate the blood and remove the serum. The use of “bloody” sera is contraindicated. Serum samples should be refrigerated as soon as possible after collection and tested within 48 hours. If the specimen is not to be tested within 48 hours after collection, the serum sample should be frozen at 0ºC or lower. Do not heat-inactivate serum and avoid repeated freezing and thawing of samples. Vortex (mix well) samples, controls, and calibrator before using.
Test samples and the calibrator are diluted 1:51 in the dilution buffer (5μl of sera + 250μl of dilution buffer)
The Negative and Positive Controls are ready to use; do not dilute.
All Reagents must be at room temperature before beginning the assay.
MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes capable of delivering 5µl, 50µl, and 250µl
Squeeze bottle for washing strips or automated plate washer (see procedural notes)
Distilled or reagent grade water and graduated cylinder
Tubes for sample and calibrator dilution
Absorbent paper
A dual wavelength (bichromatic) ELISA plate reader with a 450nm and a 620 to 650nm filter. If a bichromatic reader is not available, a single wavelength ELISA reader with a 450nm filter can be used.

PROCEDURE

See attached procedure guide. All procedures and reagents are at room temperature (15 – 25°C).

READING RESULTS

ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650nm or for a single wavelength of 450nm
Positive – Index Value of 1.0 or greater
Negative – Index Value of less than 1.0

INTERPRETATION OF RESULTS

Each Control, Calibrator, and Specimen has an OD result from the Antigen Well and the Control-Antigen Well. The OD of the Control-Antigen Well is subtracted from the OD of the Antigen Well to yield the Differential OD.

For Example:

<table>
<thead>
<tr>
<th></th>
<th>Antigen Well OD</th>
<th>Control-Antigen Well OD</th>
<th>Differential OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control:</td>
<td>0.12</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Positive Control:</td>
<td>1.82</td>
<td>0.03</td>
<td>1.79</td>
</tr>
<tr>
<td>Calibrator:</td>
<td>0.38</td>
<td>0.10</td>
<td>0.28</td>
</tr>
<tr>
<td>Specimen 1:</td>
<td>1.10</td>
<td>0.19</td>
<td>0.91</td>
</tr>
<tr>
<td>Specimen 2:</td>
<td>0.25</td>
<td>0.02</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The Index Value of the controls and specimens is obtained by dividing the Differential OD of the specimen or control by the Differential OD of the calibrator.

Calculation of the Example:

<table>
<thead>
<tr>
<th></th>
<th>Differential OD of the Calibrator</th>
<th>Index Value of Negative Control</th>
<th>Index Value of Positive Control</th>
<th>Index Value of Specimen 1</th>
<th>Index Value of Specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.28</td>
<td>0.08/0.28</td>
<td>1.79/0.28</td>
<td>0.91/0.28</td>
<td>0.23/0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.29 Index</td>
<td>6.39 Index</td>
<td>3.25 Index</td>
<td>0.82 Index</td>
</tr>
<tr>
<td></td>
<td>An Index Value of <strong>1.0 or greater</strong> is considered Positive.</td>
<td>An Index Value of <strong>less than 1.0</strong> is considered Negative.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Negative Index Values are considered to be **0.00**

If using the SMART-Calc Excel Program, just enter the OD value from the reader into the appropriate well on the Excel grid and SMART-Calc will perform all of the calculations automatically.

QUALITY CONTROL

The use of controls allows validation of the test. The results should not be used if a control, or the calibrator, is out of range. A run is valid if all three of the following conditions are met:

- **Negative Control** - Index Value of less than 0.60
- **Positive Control** - Index Value between 1.50 and 6.50
- **Calibrator** - The Differential OD must be greater than 0.00
EXPECTED VALUES
The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure; therefore, Negative specimen results should be reviewed in relation to a possible exposure date. All Positive specimen results should be confirmed by an alternate method.

TROUBLESHOOTING
Negative Control has an Index greater than 0.59
   *Suggestion:* wash more vigorously. Remove excessive liquid from the wells by slapping plate, well side down, against an absorbent towel. Do not allow test wells to dry out.
Positive Control has an Index Value of less than 1.50 or more than 6.50
   *Suggestion:* check the three “T’s”: Time, Temperature and Technique. Time: insure that the timing on the incubation stages is adhered to. Temperature: temperatures above 25°C may adversely affect the assay; Technique: check all pipettes to insure that they are properly delivering the correct volume to produce a 1:51 dilution of the calibrator and specimens. Also check your wash procedure to insure vigorous washing and removal of excess wash buffer.

PROCEDURAL NOTES
The kit Calibrator and Controls must be included on each plate tested.
Allow all reagents and samples to come to room temperature before testing. It is normal for the concentrated wash buffer to crystallize when cold. The crystals will re-dissolve once the solution returns to room temperature.
Do not use reagents beyond the expiration date printed on the label.
The dropper tips are removable from the reagent bottles to allow pipetting of reagents.
Do not inter-mix conjugates, calibrators, controls, or coated plates between different kits or different lot numbers of the same kit: these components are balanced to work together as a unit. The wash buffer, substrate, stop reagent, and dilution buffer are universal reagents and can be inter-changed between all SMART kits.
There are several types of automated plate washers available. If using an automated plate washer, you will need to validate the performance of your particular washer on the SMART assays. This can be done as simply as performing a side-by-side comparison of results achieved by manual washing versus automated washing. At the conclusion of each wash cycle, invert the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.

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A graduate of the SMART program
EIA PROCEDURE GUIDE
Epizootic Diarrhea of Infant Mice (Mouse Sera), Catalog #SMART-M19

INITIAL SETUP

1. Bring one bottle of the 20x Wash Solution to 500ml with distilled water. **MIX WELL**
2. Prepare the plate map. The columns alternate between black-ringed Antigen wells and red-ringed Control-Antigen wells. Each Control, Calibrator and specimen requires two wells: an Antigen coated well and a Control-Antigen coated well.

<table>
<thead>
<tr>
<th>Well Location</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 &amp; A2</td>
<td>Pos Control</td>
</tr>
<tr>
<td>B1 &amp; B2</td>
<td>Neg Control</td>
</tr>
<tr>
<td>C1 &amp; C2</td>
<td>Calibrator</td>
</tr>
<tr>
<td>D1 &amp; D2</td>
<td>Specimen #1</td>
</tr>
<tr>
<td>E1 &amp; E2</td>
<td>Specimen #2</td>
</tr>
<tr>
<td>F1 &amp; F2</td>
<td>Etc.</td>
</tr>
</tbody>
</table>

3. Controls are ready to use, do not dilute
4. Set up calibrator and specimen dilution tubes according to the plate map.
5. Prepare 1:51 dilution of the calibrator and specimens as follows:
   - Add 250\(\mu\)l of Dilution Buffer to all dilution tubes
   - Add 5\(\mu\)l of each specimen or calibrator to appropriate tube. **MIX WELL**

SERUM INCUBATION STAGE

6. Break-off the required number of Antigen and Control-Antigen wells and place into the plate holder. (Unused wells must be kept sealed in a dry environment)
7. Add 50\(\mu\)l of the pos and neg controls to the appropriate wells.
8. Transfer 50\(\mu\)l of each specimen and calibrator dilution tube to the appropriate wells.
9. Incubate at room temperature for **30 MINUTES**.

WASH STAGE

10. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
11. Repeat the above step four more times for a total of **5 WASHES**.
12. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.

CONJUGATE INCUBATION STAGE

13. Add 50\(\mu\)l (1 drop) of conjugate to each well.
14. Incubate at room temperature for **30 MINUTES**.

WASH STAGE

15. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
16. Repeat the above step four more times for a total of **5 WASHES**.
17. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.

SUBSTRATE STAGE

18. Add 50\(\mu\)l (1 drop) of CHROMOGEN to each well.
19. Incubate at room temperature for **10 MINUTES**.
20. **DO NOT DUMP AFTER THIS INCUBATION PERIOD**.

STOP STAGE

21. Add 50\(\mu\)l (1 drop) of STOP SOLUTION to each well.
22. Brace the plate with one hand and gently tap along the opposite side of the plate with the other to evenly distribute the Stop Solution in each well.
23. Read the plate at bichromatic 450/620-650nm wavelengths (or a single wavelength of 450nm) within 30 minutes of adding the Stop Solution