**DENGUE ELISA IgM CAPTURE**

For research use only

M1018: Test for the detection of IgM antibodies to dengue virus in human serum. 96 tests.

**PRINCIPLE OF THE TEST:**
The ELISA method is based upon the capture of IgM in the sample with anti-IgM antibodies adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. Then the antigen labeled with peroxidase react with the IgM captured, and the unbound is eliminated by washing; bound antigen is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

**KIT FEATURES:**
All reagents, except for the washing solution and the antigen, are supplied ready to use. Serum dilution solution is colored to help in the performance of the technique. Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

**KIT CONTENTS:**
- VIRCELL IgM CAPTURE PLATE: 1 96-wells plate coated with anti-IgM antibodies (μ-specific).
- VIRCELL IgM POSITIVE CONTROL: 1.5 ml of positive control containing Proclin. Ready to use.
- VIRCELL IgM CUT OFF CONTROL: 2 vials with 1.5 ml of cut off control containing Proclin. Ready to use.
- VIRCELL IgM NEGATIVE CONTROL: 1.5 ml of negative control containing Proclin. Ready to use.
- VIRCELL DENGUE ANTIGEN: 5 vials of inactivated dengue virus type 1 strain Hawaii, type 2 strain New guinea C, type 3 strain H87 and type 4 strain H241, lyophilized. It contains Proclin.
- VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
- VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
- VIRCELL WASH BUFFER: 50 ml of 20x washing solution: a phosphate buffer containing Tween®-20 and Proclin.
- VIRCELL DENGUE CONJUGATE SOLUTION: 17 ml of a buffered solution for reconstitution of lyophilized conjugate, containing a peroxidase labeled monoclonal anti-dengue antibody. It contains Proclin.

Store at 2-8°C and check expiration date.

Materials required but not supplied:
- Precision micropipettes 5 and 100 μl.
- Eight channel micropipette 100 μl.
- ELISA plate washer.
- Thermostatted incubator/water bath.

**ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter. Distilled water. Alternatively, an ELISA automated processor.**

**STORAGE REQUIREMENTS:**
Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

**STORAGE OF REAGENTS ONCE OPENED:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x washing solution</td>
<td>4 months at 2-8°C</td>
</tr>
<tr>
<td>Reconstituted antigen-conjugate complex</td>
<td>2 months at 2-8°C</td>
</tr>
<tr>
<td>Rest of reagents</td>
<td>Refer to package label for expiration date(at 2-8°C)</td>
</tr>
</tbody>
</table>

**STABILITY AND HANDLING OF REAGENTS:**
Handle reagents in aseptic conditions to avoid microbial contaminations. Do not let the plate dry between washing and reagent addition. Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate. Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

**RECOMMENDATIONS AND PRECAUTIONS:**
1. For research use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the serum dilution, washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL CAPTURE ELISA references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Serum dilution solution, plate, conjugate, reconstitution solution and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The reconstituted antigen-conjugate complex contains inactivated dengue antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for waste disposal.
7. Substrate solution may be irritant to skin and mucus. In case of contact with this solution, rinse thoroughly with water.
and seek medical attention. For further information a Material Safety Data Sheet is available.

**SPECIMEN COLLECTION AND HANDLING:**
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

**PRELIMINARY PREPARATION OF THE REAGENTS:**
The washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

Antigen-conjugate complex must be prepared in advance, at least one hour before use. Add 3 ml of conjugate solution to one vial of lyophilized antigen. Let stand for one minute to allow rehydration and mix thoroughly by vortexing. The reconstituted complex antigen-conjugate can be used for 2 months if stored at 2-8°C.

**ASSAY PROCEDURE:**
1. Set incubator/water bath to 37±1°C.
2. Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3. Shake all components.
4. Remove the plate from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed.
5. Prepare a 1/20 dilution of serum samples in tubes prepared by adding 5 µl of sample to 95 µl of sample dilution solution (1/20 dilution).
6. Add 80 µl of sample dilution solution into all wells except those assigned to controls. Add 20 µl of the 1/20 dilutions of serum samples, 100 µl of positive control, 100 µl of cut off control (cut off in duplicate) and 100 µl of negative control into the corresponding wells.
7. Cover with a sealing sheet and incubate at 37±1°C for 60 min.
8. Prepare the antigen-conjugate complex according to “Preliminary preparation of reagents”.
9. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution per well. Drain off any remaining liquid.
10. Immediately add 100 µl of reconstituted antigen-conjugate into each well.
11. Cover with a sealing sheet and incubate in incubator/water bath at 37±1°C for 60 min.
12. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution per well. Drain off any remaining liquid.
13. Immediately add 100 µl of substrate solution into each well.
14. Incubate at room temperature for 20 min. protected from light.
15. Add immediately 50 µl of stopping solution into all wells.
16. Read with an spectrophotometer at 450/620 nm within 1 hour of stopping.

**INTERNAL QUALITY CONTROL:**
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. The control material is traceable to reference sera panels internally validated.

**VALIDATION PROTOCOL FOR USERS:**
Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.

Optical densities (O.D.) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

<table>
<thead>
<tr>
<th>Control</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Negative control</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Cut off control</td>
<td>&gt;0.55</td>
</tr>
<tr>
<td></td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF RESULTS:**
Calculate the mean O.D. for cut off serum.

**Antibody index=(sample O.D./ cut off serum mean O.D.) x 10**

<table>
<thead>
<tr>
<th>Index</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>&lt; 9</td>
<td>Negative</td>
</tr>
<tr>
<td>9-11</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt;11</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Samples with equivocal results must be retested and/or a new sample obtained for confirmation. Samples with indexes below 9 are considered as not having IgM specific antibodies against dengue. Samples with indexes above 11 are considered as having IgM specific antibodies against dengue.

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SYMBOLS USED IN LABELS:

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUO</td>
<td>For research use only</td>
</tr>
<tr>
<td></td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td></td>
<td>Store at x-yºC</td>
</tr>
<tr>
<td></td>
<td>Contains sufficient for &lt;X&gt; test</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td></td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>WELLS</td>
<td>&lt;X&gt; wells</td>
</tr>
</tbody>
</table>

For any question please contact:
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