Version 18-04/24

User's Manual

Japanese Encephalitis IgM ELISA Kit

REF **DEIA1978**

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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.

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PRODUCT INFORMATION

Intended Use

The Japanese Encephalitis (JE) ELISA IgM Antibody Capture ELISA (MAC-ELISA) test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA assay system for the detection of IgM antibodies in human serum to JEV-derived recombinant antigen (JERA). For Research use only not for use in diagnostic procedures.

General Description

Exposure to JEV causes a disease with a number of symptoms including encephalitis. JE ELISA IgM Antibody Capture ELISA (MAC-ELISA) employs a recombinant antigen called JERA, which can be used as a rapid serological marker for JEV infection. The JERA protein is a recombinant antigen, which consists of a stretch of peptides from different parts of the JEV.

Principles of Testing

The JE ELISA IgM Antibody Capture ELISA (MAC-ELISA) consists of one enzymatically-amplified "two-step" sandwich-type immunoassay. In this assay, JE ELISA Negative Control (represents non-reactive serum), JE ELISA IgM Positive Control (represents reactive serum), and unknown serum samples are diluted with Sample Dilution Buffer, then incubated in microtitration wells which have been coated with anti-human IgM antibodies. This is followed by incubation with both JEVderived recombinant antigen (JERA) and Normal Cell Antigen (NCA) separately. After incubation and washing, the wells are treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the JERA and the control wells accurately determines whether antibodies to JEV are present.

Reagents And Materials Provided

1. Coated Microtiter Strips for Human IgM: Strip holder in foil pouch, containing 96 polystyrene microtiter wells coated with antibody to human IgM in each well. Store at 2-8˚C until ready to use. The anti-human IgM coated wells are used to capture IgM antibodies from human samples.
2. Sample Dilution Buffer for IgM Type A: One bottle, 25 mL, for serum dilution prior to use in assay. Store at 2- 8˚C until ready to use. Note: If any precipitate is seen, vortex the tube very well to obtain a homogeneous solution before use.
3. JE Negative Control: One vial, 50 µL. The JE Negative Control will aid in monitoring the integrity of the kit. Store at 2-8˚C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
4. JE IgM Positive Control: One vial, 50 µL. The JE IgM Positive Control will aid in monitoring the integrity of the kit. Store at 2-8˚C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.

5. Ready to Use JE Antigen (JERA) for IgM: One bottle, 5 mL of a pre-diluted JERA solution. Store at 2-8˚C until ready to use.
6. Ready to Use normal cell antigen (NCA) for JE IgM: One bottle, 5 mL of a prediluted NCA solution. Store at 2-8˚C until ready to use.
7. Ready to Use Enzyme Conjugate-HRP for JE IgM: One bottle, 9mL of a prediluted HRP conjugated flavivirus reactive monoclonal antibody (mAb). Store at 2- 8˚C until ready to use. Note: The conjugate should be kept in a light-protected bottle at all times as provided.
8. 10X Wash Buffer: One bottle, 120 mL of Wash Buffer to be used in all the washing steps of this procedure. Store at 2-8˚C until ready to use.
9. EnWash: One bottle, 20 mL of EnWash to be used in between the washing steps after the addition of enzyme conjugate-HRP of this procedure. Store at 2-8°C until ready to use.
10. Liquid TMB Substrate: One bottle, 12 mL of liquid substrate. Store at 2-8˚C until ready to use. Note: The substrate should be kept in a light -protected bottle at all times as provided.
11. Stop Solution: One bottle, 9 mL to be used to stop the reaction. Store at 2-8˚C until ready to use. Caution: strong acid, wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

Materials Required But Not Supplied

1. ELISA Spectrophotometer capable of absorbance measurement at 450 nm
2. Biological or High-Grade Water
3. Vacuum Pump
4. Plate Washer
5. 37˚C (±2°C) Incubator
6. 1-10 µL Single-Channel Pipettors, 50-200 µL Single-and Multi-Channel Pipettors
7. Polypropylene tubes
8. Parafilm or similar plate cover
9. Timer
10. Vortex

Storage

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Specimen Collection And Preparation

1. Human serum must be used with this assay. Whole blood or plasma cannot be tested directly. Note: CSF can be used. However, our kit has not been tested or optimized with CSF. Before using the DAI kit, one has to optimize the CSF system.
2. Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
3. Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for

prolonged periods.

4. Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8˚C for up to 7 days. To maintain long-term longevity of the serum, store at -20˚C or lower. Avoid repeated freezing and thawing of samples.
5. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
6. If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
7. Do not use sera if any indication of growth is observed.
8. CSF application: CSF should be run using undiluted samples. If there is not enough volume to test, the CSF samples may be diluted 1:2 or higher using the Sample Dilution Buffer provided. However, one needs to optimize the proper dilution factor. The rest of the process is the same as described for serum. Note: It is necessary to validate the CSF system in a laboratory before using unknown samples.

Reagent Preparation

1. Preparation of 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 mL 10X wash buffer with 1080 mL high-grade water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at room temperature for up to 6 months. Check for contamination prior to use.
2. Microtitration Wells: Select the number of coated wells required for the assay. The remaining unused wells should be placed back quickly into the pouch and stored at 2-8°C until ready to use or expiration.

Assay Procedure

Bring all kit reagents and specimens to room temperature (20-25˚C) before use. Thoroughly mix the reagents and samples before use by gentle inversion.

1. Positive and negative controls should be assayed in duplicate for both JERA and NCA portions of assay. Unknown serum samples to be tested can be assayed singly or in duplicate but must be assayed for both JERA and NCA portions of assay. Refer to flow chart at the end of this section for illustration of this procedure. Up to forty-four test specimens can be tested on one 96-well plate.
2. Mark the microtitration strips to be used.
3. Dilute test sera and controls to 1/100 using the provided Sample Dilution Buffer. Use small polypropylene tubes for these dilutions and at least 4 µL of sera and positive and negative controls. For example: 4 µL serum plus 396 µL of Sample Dilution Buffer to make 1/100 dilution.
4. Apply the 50 µL/well of 1/100 diluted test sera, JE ELISA Negative Control, and JE ELISA IgM Positive Control to the plate by single or multi-channel pipettor as appropriate. An exemplary arrangement is shown below.

Example for Serum Sample Application												
	1	2	3	4	5	6	7	8	9	10	11	12
A	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
B	JE Negative Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
C	JE IgM Positive Control	S#23	S#25	S#27	S#29	S#31	S#33	S#35	S#37	S#39	S#41	S#43
D	JE IgM Positive Control	S#24	S#26	S#28	S#30	S#32	S#34	S#36	S#38	S#40	S#42	S#44
E	JE IgM Positive Control	S#24	S#26	S#28	S#30	S#32	S#34	S#36	S#38	S#40	S#42	S#44
F	JE IgM Positive Control	S#23	S#25	S#27	S#29	S#31	S#33	S#35	S#37	S#39	S#41	S#43
G	JE Negative Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
H	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21

5. Cover the plate with parafilm or similar cover just on the well opening surface, so the bottom of the plates is not covered. Note: This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.
6. Incubate the plate at 37˚C for 1 hour in an incubator. Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.
7. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µL per well in each wash cycle.
8. Add 50 µL/well of JERA into row A-D and 50 µL/well of NCA into row E-H by multi-channel pipettor. An exemplary application for JERA and NCA is shown below.

Example for JE Antigens Application												
	1	2	3	4	5	6	7	8	9	10	11	12
A	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
B	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
C	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
D	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
E	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
F	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
G	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
H	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA

9. Cover the plate with parafilm or similar cover just on the well opening surface, so the bottom of the plate is not covered (see step 5).

10. Incubate the plate at 37˚C for 1 hour in an incubator.
11. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µL per well in each wash cycle.
12. Add 50µL/well of ready to use Enzyme-HRP conjugate into all wells by multichannel pipettor.
13. Cover the plate with parafilm or similar cover just on the well opening surface, so the bottom of the plate should not be covered (see step 5).
14. Incubate the plate at 37˚C for 1 hour in an incubator in darkness.
15. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µL per well in each wash cycle.
16. Add 150 µL/well of EnWash into all wells by multi-channel pipettor.
17. Incubate the plate at room temperature for 5 minutes without any cover on the plate.
18. After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µL per well in each wash cycle.
19. Add 75 µL/well of Liquid TMB substrate into all wells by multi-channel pipettor.
20. Incubate the plate at room temperature in a dark place (or container) for 10 minutes without any cover on the plate.
21. After the incubation, add 50 µL/well of Stop solution into all wells by multichannel pipettor.
22. Within 5 minutes, read the RAW OD 450 value with a Microplate reader. Please make sure the Microplate reader DOES NOT SUBTRACT OR NORMALIZE ANY BLANK VALUES OR WELLS.

Quality Control

Each kit contains positive and negative control sera to ensure assay performance. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cut-off. The test is invalid and must be repeated if the ISR values of either of the controls do not meet the specifications. Acceptable Immune Status Ratio (ISR) values for these controls are found on the specification table below. If the test is invalid, patient results cannot be reported. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only. Applicable for raw spectrophotometric readings only. Do not subtract any background.

Calculation of the Negative Control: Calculate the mean JE ELISA Negative Control values with JERA and with the Control antigen, then calculate the JERA/NCA ratio. Any JE Negative Control JERA/NCA ratio greater than 2.8 indicates that the test procedure must be repeated.

Example: JE Negative Control

	OD	
	JERA	NCA
No 1	0.188	0.129
No 2	0.192	0.125
Total	0.380	0.254

Averages (JERA) = 0.380 ÷ 2 = 0.190
(NCA) = 0.254 ÷ 2 = 0.127

Calculate the JERA/NCA ratio: 0.190 ÷ 0.127 = 1.50

Calculation of the Positive Control: Calculate the mean JE ELISA IgM Positive Control values with JERA and with the NCA, then calculate the JERA/NCA ratio. Any JE IgM Positive Control JERA/NCA ratio less than 6.0 indicates that the test procedure must be repeated.

Example: JE IgM Positive Control

	OD	
	JERA	NCA
No 1	1.035	0.105
No 2	1.055	0.115
Total	2.090	0.220

Averages (JERA) = 2.090 ÷ 2 = 1.045
(NCA) = 0.220 ÷ 2 = 0.110

Calculate the JERA/NCA ratio: 1.045÷ 0.110 = 9.5

Interpretation Of Results

Calculation of the Immune Status Ratio (ISR): Calculate the average JERA and NCA OD450nm values for each of the controls. For test samples run in singlet, obtain the individual JERA and NCA values. Determine the Immune Status Ratio (ISR) values for the controls and all test sera by dividing the JERA OD450nm /NCA OD450nm. The assay performance is deemed valid when the ISR for the positive control is greater than 6.0, and the ISR for the negative control is less than 2.8.

Selection of the Cut-off: The cut-off was selected using values from a small set of field data and is an estimate only.

Interpretation of Results: The table below shows how the results should be interpreted.

ISR	Results	Interpretation
<4.0	Negative	No detectable IgM antibody by the ELISA test
4-6	Equivocal	Need confirmatory test
>6.0	Positive	Indicates presence of detectable IgM antibody. Recommend supplemental confirmatory testing.

Sensitivity

Serological Sensitivity: 31/31, or 100%

Specificity

Serological Specificity: 0/196, or 100%

Precautions

1. A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
2. Do not use any component beyond the kit's expiration date.
3. Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
4. Some reagents may form a slight precipitate, mix gently before use.
5. Avoid repeated freezing and thawing of the serum specimens to be evaluated.
6. This test must be performed on freshly diluted serum only. The use of whole blood, plasma or other specimen matrix has not been validated. Do not store serum diluted in sample dilution buffer.
7. Do not mix various lots of any kit component within an individual assay.
8. Treat all sera as infectious material. Do not heat-inactivate test sera.
9. All reagents must be equilibrated to room temperature (20-25˚C commencing the assay. The assay will be affected by temperature changes.

Limitations

1. Since this is an indirect screening method, the presence of false positive and negative results must be considered.
2. All reactive samples must be evaluated by a confirmatory test.
3. The reagents supplied in this kit are optimized to measure JERA reactive antibody levels in serum.
4. Serological cross-reactivity across the flavivirus group is common. Certain sera from patients infected with Dengue, West Nile, and Saint Louis virus may give false positive results. Therefore any JE positive sera must be confirmed with other tests.
5. In areas where JE and dengue are co-existent, JE positive samples should also be assayed for dengue reactivity. Samples with borderline JE positivity and medium to high dengue reactivity could be suspected for dengue infection and require further confirmatory assays.
6. The assay performance characteristics have not been established for visual result determination.
7. Results from immunosuppressed patients must be interpreted with caution.
8. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.