



### AccuDiag™ Japanese Encephalitis IgM ELISA Kit

REF 8403

PIC ID8403XU1

IVD See External Label 2°C 8°C 96 Tests

Japanese Encephalitis IgM ELISA	
Principle	Sandwich ELISA
Detection	Qualitative
Sample	4 µL serum/plasma
Incubation Time	195 minutes
Sensitivity	100%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

#### PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

#### INTENDED USE

The Japanese Encephalitis (JE) ELISA IgM Antibody Capture ELISA (MAC-ELISA) test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA system for the detection of IgM antibodies in human serum to JEV-derived recombinant antigen (JERA) (1-4).

#### SIGNIFICANCE AND SUMMARY

Exposure to JEV causes a disease with a number of symptoms including encephalitis (5-8). 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.

#### ASSAY PRINCIPLE

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 JEV-derived 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.

#### SPECIMEN COLLECTION & PREPARATION

- 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 Diagnostic Automation Inc. kit, one has to optimize the CSF system.*
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- 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.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera if any indication of growth is observed.

#### REAGENTS

**Warning: Do not use any reagents where damage to the packaging has occurred.**

##### Materials provided with the kit

The JE ELISA IgM Antibody Capture ELISA (MAC-ELISA) kit contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each.

The kit contains the following reagents:

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.

- 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.
- 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.
- 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.
- Ready to Use normal cell antigen (NCA) for JE IgM:** One bottle, 5 mL of a pre-diluted NCA solution. Store at 2-8°C until ready to use.
- Ready to Use Enzyme Conjugate-HRP for JE IgM:** One bottle, 9mL of a pre-diluted 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.
- 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.
- Wash Solution:** One bottle, 20 mL of Wash Solution 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.
- 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.
- 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 provided

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

### REAGENT PREPARATION

- 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.
- 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

This kit has not been optimized by Diagnostic Automation Inc. for use with any particular automated ELISA processing system. Use with an automated ELISA processing system will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes of reagents may be required.

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.

**Note: All serum, including the experimental, cannot be repeatedly thawed and frozen. For long-term storage, sera should be further aliquoted in a smaller volume and stored at -20°C or lower. Always quick spin serum sample contained in vials or tubes to collect sample at the bottom.**

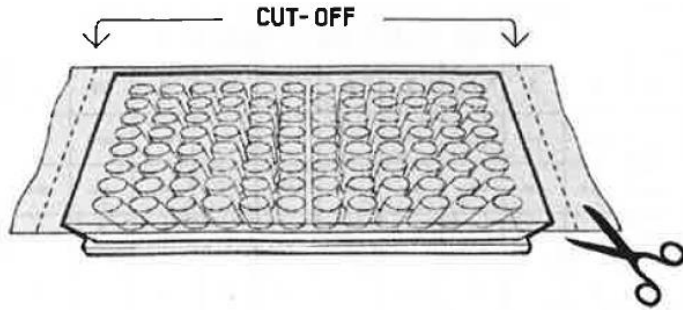
- Positive and negative controls should be assayed in duplicate for both JERA and NCA portions of assay. Unknown serum samples to be tested can be assayed singly or in duplicate but must be assayed for both JERA and NCA portions of assay. Refer to flow chart at the end of this section for illustration of this procedure. Up to forty-four test specimens can be tested on one 96-well plate.
- Mark the microtitration strips to be used.
- 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.
- 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
<b>A</b>	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>B</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
<b>C</b>	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
<b>D</b>	JE IgG Positive Control	S# 24	S# 26	S# 28	S# 30	S# 32	S# 34	S# 36	S# 38	S# 40	S# 42	S# 44
<b>E</b>	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
<b>F</b>	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
<b>G</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
<b>H</b>	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



- 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 ensure 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.

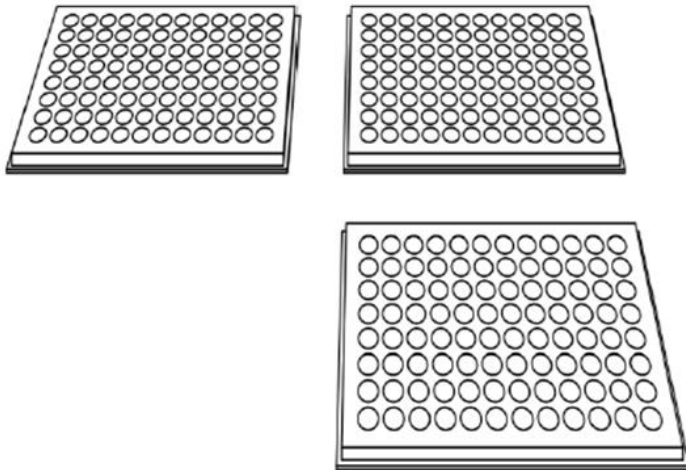


- Incubate the plate at 37°C for 1 hour in an incubator.

**Note:** Do not stack plates on top of each other. They should be spread out as single layer. This is very important for even temperature distribution. Do not use CO<sub>2</sub> or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.



INCORRECT METHOD



CORRECT METHOD

- 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.
- 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

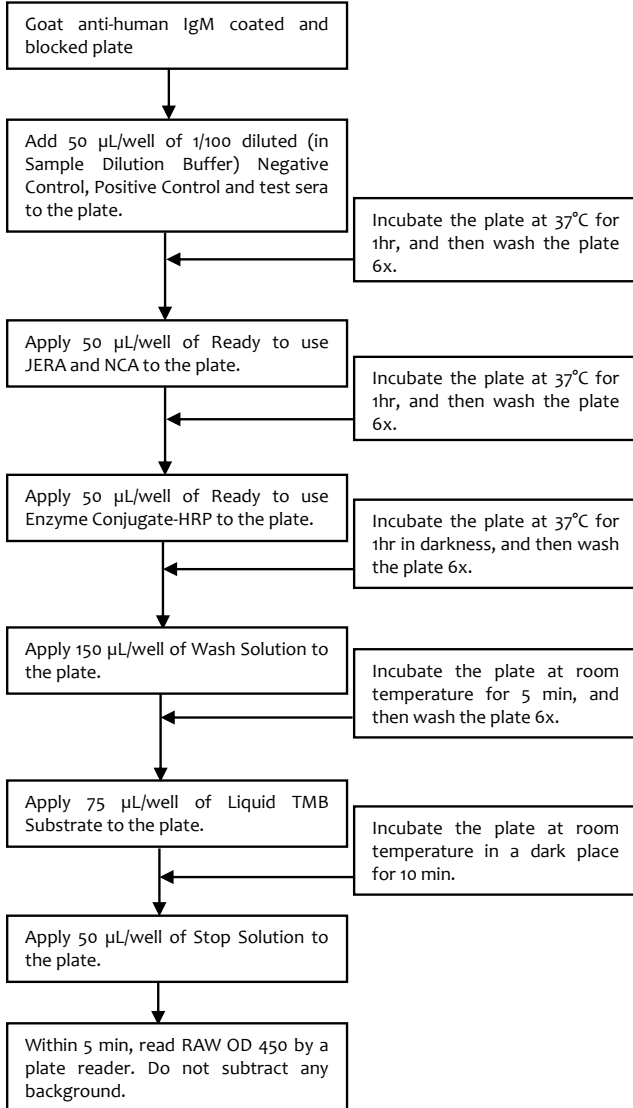
- Cover the plate with parafilm or similar cover just on the well opening surface, so the bottom of the plate is not covered (see step 5).
- Incubate the plate at 37°C for 1 hour in an incubator.
- After the incubation, wash the plate 6 times with an automatic plate washer using 1x Wash buffer. Use 300 µL per well in each wash cycle.
- Add 50 µL/well of ready to use Enzyme-HRP conjugate into all wells by multi-channel pipettor.
- 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).
- Incubate the plate at 37°C for 1 hour in an incubator in darkness.
- 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.
- Add 150 µL/well of Wash Solution into all wells by multi-channel pipettor.
- Incubate the plate at room temperature for 5 minutes without any cover on the plate.
- 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.
- Add 75 µL/well of Liquid TMB substrate into all wells by multi-channel pipettor.
- Incubate the plate at room temperature in a dark place (or container) for 10 minutes without any cover on the plate.
- After the incubation, add 50 µL/well of Stop solution into all wells by multi-channel pipettor.
- 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.

**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.



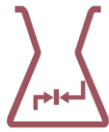
### Procedure Flowchart:



### TROUBLESHOOTING

Problem	Possible Cause	Possible Resolution	
<b>High Absorbance for JERA/NCA</b>	Incomplete washing of wells	Wells must be completely filled emptied 6 times during each wash cycle	
	Incubation times too long	Incubation times vary, please refer to the "Test Procedure" section for correct times	
	Conjugate contamination with TMB	It is recommended to use a new pipette/pipette tip each time to dispense conjugate and TMB	
	Incorrect wavelength filter	The optical density readings must be read with <b>ONLY</b> a 450nm filter. There must not be any background subtraction.	
<b>Low Absorbance for JERA/NCA</b>	Samples incorrectly diluted	Sera should be diluted 1:100 in kit's sample dilution buffer	
	Kit expiration date and storage	Verify that the kit is not expired and that components were properly stored.	
	Incorrect component used	Do not combined controls or reagents between different lots of ELISA kits	
	Component temperature	All kit components must be equilibrated at room temperature for optimal performance	
	Incubation times too short	Incubation times vary, please refer to the "Test Procedure" section for correct times	
	Incubation temperature too low or high	Verify that incubators are calibrated with NIST-traceable standard and that the temperatures are monitored	
	JERA/NCA contamination	The antigens are very susceptible to contamination. It is recommended to use a new pipette/pipette tip each time to dispense antigens. Keep the lid on the antigens unless in use. When possible, dispense antigens in a clean laminar flow hood or biological safety cabinet	
	Conjugate contamination	The conjugate are very susceptible to contamination. It is	

Problem	Possible Cause	Possible Resolution
	Incorrect component used	Do not combined controls or reagents between different lots of ELISA kits
	Samples incorrectly diluted	Sera should be diluted 1:100 in kit's sample dilution buffer
	Cross-contamination of wells	A new tip must be used for every test or control sera



Problem	Possible Cause	Possible Resolution
		recommended to use a new pipette/pipette tip each time to dispense conjugate. Keep the lid on the conjugate unless in use. When possible, dispense conjugate in a clean laminar flow hood or biological safety cabinet
	TMB contamination with Stop solution	It is recommended to use a new pipette/pipette tip each time to dispense stop solution and TMB
	Use of reagents in the wrong sequence, or omission of step(s)	Check the "Test Procedure" section and component labels prior to use
	Incorrect wavelength filter	The optical destiny readings must be read with <b>ONLY</b> a 450nm filter. There must not be any background subtraction.

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.

Example: JE Negative Control

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

$$\text{Averages (JERA)} = 0.380 \div 2 = 0.190$$

$$\text{(NCA)} = 0.254 \div 2 = 0.127$$

Calculate the JERA/NCA ratio:  $0.190 \div 0.127 = 1.50$

Any JE Negative Control JERA/NCA ratio greater than 2.8 indicates that the test procedure must be repeated.

**Calculation of the Positive Control:** Calculate JE ELISA IgM Positive Control values with JERA and with the NCA, then calculate the JERA/NCA ratio.

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

$$\text{Averages (JERA)} = 2.090 \div 2 = 1.045$$

$$\text{(NCA)} = 0.220 \div 2 = 0.110$$

Calculate the JERA/NCA ratio:  $1.045 \div 0.110 = 9.5$

Any JE IgM Positive Control JERA/NCA ratio less than 6.0 indicates that the test procedure must be repeated.

The results in the table below must be obtained in order that the results of the run may be reported. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor (For Assay Verification)	Tolerance
Mean Negative Control OD in JERA	< 0.300
Mean Positive Control OD in JERA	> 0.600
Positive Control Immune Status Ratio (ISR)	> 6.000
Negative Control Immune Status Ratio (ISR)	< 2.800

### RESULTS

**Calculation of the Immune Status Ratio (ISR):** Determine the average of the duplicates for each control sample with the JERA and with the NCA. For each control and test sample, calculate the ratio of the JERA OD to the NCA OD (JERA/NCA ISR). The ISR for the positive control should be greater than 6.0, while the ISR for the negative control should be 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

### 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 CLSI C24 and 42 CFR 493.1256 for guidance on appropriate QC practices. The

### LIMITATIONS OF THE ASSAY

- For *in vitro* diagnostic use only. Not for sale or distribution in the United States of America.
- Since this is an indirect screening method, the presence of false positive and negative results must be considered.
- The reagents supplied in this kit are optimized to measure JERA reactive antibody levels in serum.
- This kit has not been optimized for vaccine-induced seroconversion studies.



### PRECAUTIONS

#### BEFORE STARTING:

- 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.
- Do not use any component beyond the kit's expiration date.
- 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.
- Some reagents may form a slight precipitate, mix gently before use.
- Avoid repeated freezing and thawing of the serum specimens to be evaluated.
- 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.
- Do not mix various lots of any kit component within an individual assay.
- Treat all sera as infectious material.
- **Do not heat-inactivate test sera.**
- All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Cover working area with disposable absorbent paper.

#### DURING THE ASSAY:

- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do so may cause erroneous results with those unused microwells.
- During dilution of the controls and test sera in sample dilution buffer it is **critical** that a **new pipette tip** be used for each sample to avoid cross-contamination.
- All reagents are susceptible to contamination; thus, it is advisable to dispense reagents directly from bottles using clean pipettes or by carefully pouring. Pipettes should be used **only once** to avoid contamination of the components.
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stop solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents, especially for the Ready to Use Enzyme Conjugate-HRP for JE IgM.
- Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP. The TMB substrate is clear; if it appears **blue**, do not use as it may be contaminated.
- NIST-traceable standards should be used to calibrate the thermometers used to monitor the temperature of the incubator. It is important that the incubator is set at the right temperature.

#### WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

- All human source materials used in the preparation of controls have been heat-inactivated and have tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency, therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommends that potentially infectious agents be handled at the Biosafety Level 2.

#### CHEMICAL HAZARD:

- Safety Data Sheets (SDS) are available for all components of this kit. Review all appropriate SDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

### REFERENCES

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### MANUFACTURER AND BRAND DETAILS

<b>ISO 13485:2016</b>	
Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA	
<b>Date Adopted</b>	2023-09
<b>Brand Name</b>	AccuDiag™
<b>REF</b> 8403	AccuDiag™ - Japanese Encephalitis IgM ELISA
<b>PIC</b>	ID8403XU1
<b>EC REP</b>	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a>
<b>Revision Date: 2019-07-09</b>	