DRG® Mitochondria IgG,A,M (EIA-4143)

USA: RUO

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INTENDED USE

The DRG Mitochondria Enzyme-Linked Immunosorbent Assay (ELISA) is intended for detection of antibodies to mitochondria in human sera. The assay is to be used to detect antibodies in a single serum specimen. This kit is intended for Research Use Only.

PRINCIPLE OF THE TEST

The DRG Mitochondria test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgM, and IgA antibodies to mitochondria antigens. Purified Mitochondria antigens are attached to a solid phase microassay well. Diluted test sera are added to each well. If the antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG, M, A, is added to each well. If antibody is present the conjugate will bind to the antigen-antibody complexes. After incubation the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the donor specimen.

KIT COMPONENTS

1. Mitochondria antigen coated microassay plate: 96 well, provided with a strip holder and stored in a foil bag with desiccant and humidity indicator card.
2. Wash Buffer (20x concentrate) Type II: One bottle, 50 mL. Contains buffer and Tween 80, and 0.1% proclin as a preservative.
3. Serum Diluent: One bottle, 30 mL. Contains buffer, BSA and Tween 80 and 0.1% proclin as a preservative.
4. Conjugate: One bottle, 15 mL. Contains horseradish peroxidase conjugated anti-human IgG, IgM and IgA in a buffer.
5. Chromogen/Substrate: One bottle, 15 mL. Contains 3, 3', 5, 5' - tetramethylbenzidine (TMB).
6. Stop Solution: One bottle, 15 mL. Contains a H_2SO_4 solution.
7. High Positive Control: One vial, 0.4 mL, human serum containing 0.1% sodium azide and 0.01% pen/strep as preservatives, with antibodies that react strongly with the antigen. Established range printed on vial label.
8. Negative Control: One vial, 0.4 mL, human sera containing 0.1% sodium azide and 0.1% pen/strep as preservatives, with antibodies that do not react with the antigen. Established range printed on vial label.
9. Low Positive Control: One vial, 0.4 mL, human serum containing 0.1% sodium azide and 0.01% pen/strep as preservatives, with antibodies that react weakly with the antigen. Established range printed on vial label.
10. Calibrator: One vial, 0.4 mL, human containing 0.1% sodium azide and 0.01% pen/strep as preservatives, with antibodies that react with the antigen used to calibrate the assay. Kit specific Correction Factor printed on vial label.

REAGENT STORAGE CONDITIONS

1. All kit components that are stored at their recommended storage conditions are stable until the expiration date on their label. Do not use past their expiration date.
2. Antigen coated wells. Unused strips should be immediately resealed in the foil bags with desiccant and humidity indicator card and returned to storage at 2-8° C. If the bag is resealed with tape the wells are stable for 30 days. If the bag is resealed with a heat sealer the wells are stable until their expiration.
3. All other reagents are stored at 2-8° C in their original containers.

4. Store 1X (diluted) Wash Buffer at room temperature (21° to 25° C) for up to 5 days, or 1 week between 2° and 8° C.

PRECAUTIONS

1. Each donor unit used in the preparation of the Calibrator and Controls was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative.
2. Certain reagents in this kit contain sodium azide for use as a preservative. Azides may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water to minimize azide build up.
3. This product is for RESEARCH USE only.
4. Reagents contain preservatives which may be toxic if ingested.
5. Do not pipette by mouth. Avoid contact of reagents and donor specimens with skin or mucous membranes.
6. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with copious quantities of water.
7. Avoid splashing or generation of aerosols.
8. Do not use heat inactivated sera.
9. Do not mix or interchange reagents between lots of kits or from other manufacturer.
10. Do not dilute or adulterate kit reagents.
11. Do not cross contaminate reagents or specimens.
12. Do not use Chromogen/Substrate Solution if it has begun to turn blue.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Do not vary reagent and incubation temperatures above or below room temperature (21 - 25° C).
15. Washing is important. Improperly washed wells will give erroneous results. Do not allow the well to dry out between incubations.

SPECIMEN COLLECTION

1. Aseptically collect blood samples by venipuncture and prepare serum using accepted technique (12).
2. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
3. Sera may be stored up to five days at 2-8° C. If a further delay in testing is needed store frozen at -20 to -70° C in a non-defrosting freezer. Avoid multiple freeze/thaw of donor samples.
4. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
5. Do not heat inactivate sera.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels and test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water, CAP Type 1 or equivalent.
9. Timer capable of measuring to an accuracy of +/- 1 second.
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL H_20).

11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the operators' manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

PREPARATION OF REGENTS

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25° C) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer to 1 L with distilled and/or deionized H_20. Mix well.

GENERAL PROCEDURE

1. Determine the number of donors to be assayed. For each assay the Calibrator should be run in triplicate. Also the High Positive Control, Low Positive Control, Negative Control, and a reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations.

Example Configuration:

1A	RB	2A	Donor #2
1B	NC	2B	Donor #3
1C	Cal	2C	Donor #4
1D	Cal	2D	Donor #5
1E	Cal	2E	Donor #6
1F	HPC	2F	Donor #7
1G	LPC	2G	Donor #8
1H	Donor #1	2H	Donor #9

2. For each test serum, Calibrator and Control to be assayed prepare a 1:21 serum dilution. Add 10 µL of each serum sample to 200 µL of Serum Diluent . Mix well.
3. Remove the number of wells needed from the plate bag and arrange in a strip holder. The remaining strips should be resealed in the plate bag with dessicant/humidity indicator. The bag should be reheat sealed or rolled over and the end taped. If the color of the indicator changes from the blue to pink, the strips should not be used.
4. Transfer 100µL of the prediluted samples to the reaction wells, using a multichannel pipette. Withdraw and expel each sample at least three times to ensure proper mixing of the sample of the before transferring to the reaction plate. Use new fresh pipette tips for each sample. Add 100 uL of Serum Diluent to the reagent plate.
5. Incubate the plate at room temperature (21-25°C) for 30 minutes +/- 1 minutes.
6. Wash the reaction plate three times with 1X Wash Buffer. Shake all of the liquid out of the wells. With a wash bottle, automated or semi-automated wash sysytem, fill each well with 250-300uL Wah Buffer making sure no air bubbles are trapped in the wells. Shake all of the Wash Buffer out of the wells. Repeat the wash two or more times. A total of up to 5 washes may be necessary with automated equipment. After the last wash, shake out the Wah Buffer and remove residual Wash Buffer by tapping the plate firmly on a paper towel. The Wash Buffer can be collected in a basin and treated with 0.5% sodium hypochlorite (bleach) at the end of the day.

7. Add 100 uL Conjugate to each well of the reaction plate, including reagent blank.
8. Incubate each well of the reaction plate at room temperature (21-25)°C for 30 minutes +/- 1 minutes.
9. Repeat wash as described in Step 6.
10. Add 100 uL Chromogen/Substrate Solution to each well of the reaction plate, including reagent blank.
11. Incubate each well of the reaction plate at room temperature (21-25)°C for 15 minutes +/- 1 minutes.
12. Add 100 uL of the Stop Solution to each well, including the reagent blank, at the same rate as the Chromogen/Substrate Solution was added. Positive samples will turn from blue to yellow. Tap plate to ensure mixing. Wait a minimum or 5 minutes and read.
13. Read the plate using a spectrophotometer at a wavelength of 450nm. If dual wavelength is used, set the reference filter to 600-650nm. Measure each optical density (OD) against the reagent blank. The plate should be read within 30 minutes of assay completion.

QUALITY CONTROL

1. Calibrator and Controls must be run with each test run.
2. Reagent blank must be < 0.15 O.D. at 450 nm.
3. The mean O.D. value for the Calibrator should be ≥ 0.30 at 450 nm.
4. The index values for the High, Low, and Negative Control should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.

CALCULATIONS

1. Calibrator Value - Calculate the mean value for the Calibrator from three Calibrator determinations. If any of the three Calibrator determinations differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - To account for day to day fluctuations in assay activity due to room temperature and timing, a correction factor is determined by DRG for each lot of kits. The correction factor is printed on the Calibrator vial.
3. Cutoff O.D. Value - The Cutoff O.D. value for each assay is determined by multiplying the correction factor by the mean Calibrator value determined in step 1.
4. Index Value - Calculate an Index Value for each specimen by dividing the specimen O.D. value by the cutoff O.D. determined in step 3.

Example:	O.D.s obtained for Calibrator	= 0.38, 0.42
	Mean O.D. for Calibrator	= 0.40
	O.D. obtained for donor sera	= 0.60
	Correction factor	= 0.50
	Cutoff value	= 0.50 x 0.40 = 0.20
	Index Value	= 0.60/0.20 = 3.00

REFERENCES

1. Tan, E.M. 1982. Autoantibodies to Nuclear Antigens (ANA) : Their Immunobiology and Medicine. *Adv. Immunolo*. 33 :167-240.
2. Nakamura, R.M., and E.M. Tan. 1986. Recent Advances in Laboratory Tests and the Significance of Autoantibodies to Nuclear Antigens in Systemic Rheumatic Disease. *Clin. Lab Med.* 6:41-53.

3. McCarty, G.A., D.W. Valencia, and M.J. Fritzler. 1984. Antinuclear Antibodies : Contemporary Techniques and Clinical Application to Connective Tissue Disease. In: *Antinuclear Antibodies.* Oxford Univ. Press, New York. pp 1-95.
4. Fussey, S.P.M., S.M. West, J.G. Lindsay, et al. 1991. Clarification of the identity of the major M2 autoantigen in primary biliary cirrhosis. *Clin Sci* . 80 : 451-455.
5. Berg, P.A., et al. 1982. ATPase associated antigen (M2) : marker antigen for serological diagnosis of primary biliary cirrhosis. *Lancet II* pp 1423-1426.
6. Klein, R., et al. 1983. Use of ATPase associated antigen (M2) for detection of antimitochondria antibodies in primary biliary cirrhosis by fluorometeric immunoassay. *J Immunol Meth* 64 : 227-238.
7. Kurki, P., M. Gripenberg, P. Parlanen, and T. Helve. 1987. Screening Test for Rheumatic Disease: A Combined Enzyme Immunoassay of Rheumatoid Factors and Autoantibodies to DNA and Extractable Nuclear Antigens. *J. Clin. Pathol.* 40 : 1475-1480.
8. Geisler, C. and M. Hoier-Madsen. 1985. An Enzyme-Linked Immunosorbent Assay for Autoantibodies Against the Nuclear Protein Scl-70. *J. Immuno. Methods.* 80 : 211-219.
9. Biswas, T., F.W. Miller, Y. Takagaki, and P.H. Poltz. 1987. An Enzyme -Linked Immunosorbent Assay for the Detection and Quantitation of Anti-Jo-1 Antibody in Human Serum. *J. Immun. Meth.* 98 : 243-248.
10. Rubin, R.L. 1992. Enzyme-Linked Immunosorbent Assay for Anti-DNA and Antihistone Antibodies Including Anti-(H2A-H2B). In: *Manual of Clinical Laboratory Immunology.* N.R. Rose, et al., eds. 4th Edition. 108: 735-740.
11. CDC/NIH Guidelines : Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, 1993.
12. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture. Approved Standard NCCLS Publication, H3-A. National Committee for Clinical Laboratory Standards. Villanova, PA.