



IVD For *In Vitro* Diagnostic
Cat. No.: TM-0001E

Anti-EBV IgA ELISA

For the detection and quantitative determination of human IgA antibodies to the early antigen and nuclear antigen of Epstein-Barr virus contained in human serum or plasma.

96 Tests



FORMOSA BIOMEDICAL TECHNOLOGY CORP.

QUICK INSTRUCTION:

Preparation:

1. Bring all reagents and samples to room temperature (21-25°C).
2. Prepare 1x Washing Solution by diluting 20x concentrated Washing Solution with reagent grade water.
3. Thoroughly vortex and dilute serum samples with Serum Diluents (**1 ml Serum Diluent + 10 µl serum**).

Test Procedure:

Set enough strips on the strip holder

↓
Apply **100 µl** of blank, NC, PCs and diluted serum samples to wells

100 µl

↓
Incubate at **37°C** for **60 min**

60 min

↓
Aspirate all liquid from wells. Wash wells for 3 times by 280-300 µl Washing Solution

WASH

↓
Add **100 µl** of Conjugate to wells

100 µl

↓
Incubate at **37°C** for **30 min**

30 min

↓
Aspirate all liquid from wells. Wash wells for 3 times by 280-300 µl Washing Solution

WASH

↓
Add **100 µl** of Substrate (TMB) to wells

100 µl

↓
Incubate at **37°C** for **10 min**

10 min

↓
Add **100 µl** of Stop Solution (1N HCl) to wells

100 µl

↓
Read assay plate at 450 nm using 650 nm as reference

OD_{450/650}

MeDiPro Anti-EBV IgA ELISA Kit

(Apr/22/2005)

INTENDED USE:

The **MeDiPro anti-EBV IgA ELISA**, is an enzyme linked immunosorbent assay intended for the detection and quantitative determination of human IgA to the early antigen and nuclear antigen of the Epstein-Barr virus contained in human serum.

INTRODUCTION:

Epstein-Barr virus (EBV), a human herpes virus, is the cause of a major etiological factor in a number of human diseases, including infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma.

Nasopharyngeal carcinoma (NPC) is a malignant tumor which occurs at high frequency among Chinese living in Taiwan, Hong Kong, Singapore, Malaysia and South China. It has poor prognosis due to that majority of cases is at late stage of the disease and therefore diagnosis of NPC at early stage would be important to reduce the mortality.

Seroepidemiological data have shown that sera from NPC patients have high titers of EBV specific antibodies. A rise in titer of serum IgA to EBV viral capsid antigen (VCA) and EBV induced early antigen (EA) in patients with NPC was first reported by Henle and Henle (1976). IgA and IgG to EBV membrane antigen (MA) have also been detected in sera from NPC patients (Zhu *et al.*, 1986). Although IgA responses to VCA measured in immunofluorescent assays have generally been used to screen for NPC, the studies indicate that the IgA against EA combined with EB Nuclear Antigen-1 (EBNA-1) as measured by ELISA showed better specificity and sensitivity than the IgA responses to VCA as measured by immunofluorescence (Fones-Tan *et al.*, 1994). A soluble form of the EBV EA+EBNA-1 is the basis of the serology assay being described in this instruction manual.

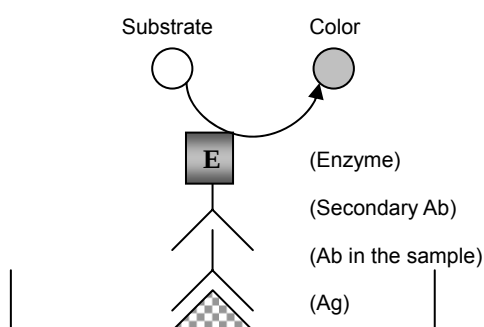
The **MeDiPro anti-EBV IgA ELISA kit**, which is developed in collaboration with Chang-Gung University, Taiwan, shows superior performance for differentiating NPC patients from normal population.

PRINCIPLES OF THE ASSAY:

1. Capture of human IgA to EBV EA+EBNA-1:

Individuals with reactivated EBV infections (e.g., NPC patients) produce antibodies to EBV in their bodies and have in their circulation, in particular IgA subclass antibodies to EBV EA+EBNA-1. Like all serology assays, the ELISA we have developed is to quantify the antibodies in serum. This is accomplished by incubating dilutions of patient serum in wells of a microtiter plate which has the antigen of interest adsorbed to it, in this case it is EBV EA+EBNA-1. The antibodies against EBV EA+EBNA-1 will bind specifically to the antigens on the microtiter plate.

The method of detecting antibody



2. Detection of bound antibodies:

After incubation for the specified time at the specified temperature, unbound antibodies are removed by aspiration and washing. The presence of bound IgA is then disclosed by using an anti-human-IgA antibodies conjugated with of horse-radish peroxidase (HRP) and the colorimetric reagent, TMB. The colorimetric result

can be determined by the microplate reader. The positivity and the concentration of antibody of the unknown samples are then calculated through an equation and a standard curve.

KIT CONTENTS:

1. ELISA Plate: One strip holder containing 8 wells x 12 strips coated with EBV EA+EBNA-1 antigens.
2. Conjugate: one bottle containing 12 ml of HRP conjugated-goat anti-human-IgA antibodies.
3. Washing Solution, 20x: one bottle, 50 ml.
4. Serum Diluents: two bottles, 50 ml each.
5. Positive Control A, 128 EU/ml: one vial, 800 µl.
6. Positive Control B, 32 EU/ml: one vial, 800 µl.
7. Positive Control C, 8 EU/ml: one vial, 800 µl.
8. Negative Control, 2 EU/ml: one vial, 800 µl.
9. Substrate (TMB): one bottle, 12 ml.
10. Stop Solution (1N HCl): one bottle, 12 ml.
11. Adhesive plate sealing film: one sheet.
12. Instruction manual.

MATERIALS REQUIRED BUT NOT PROVIDED:

1. **Micropipettes and tips** capable of accurately delivering from 25 µl to 1000 µl volumes.
2. **Multi-channel pipettes and tips** capable of accurately delivering from 25 µl to 200 µl volumes.
3. **37°C water bath or incubator.** The temperature must be within 37±2°C.
4. **Microplate reader.** The developed color should be read on an ELISA plate reader equipped with a 450 nm filter and a 650 nm reference filter.

PRECAUTIONS:

1. Safety considerations
 - 1) **For professionals and *in vitro* diagnostic use only.**
 - 2) Please refer to the manufacturer's safety data sheet and the product labeling for information on potentially hazardous components.
 - 3) Human source material please handle assay specimens, positive and negative controls as if they are capable of transmitting an infectious agent: Each donor unit used in the preparation of the controls was tested by approved methods for the presence of antibody to human immunodeficiency virus (HIV), hepatitis C virus (HCV) as well as hepatitis B surface antigen (HBsAg) and found to be negative. Because no test method can offer complete assurance that HIV, HCV, HBV or other infectious agents are absent, these materials should be handled with good laboratory practice to avoid skin contact or ingestion.
 - 4) Do not pipette by mouth. Avoid contact with skin and mucous membranes. Avoid splashing and generating aerosols.
 - 5) Do not eat, drink, or smoke in areas in which specimens or kit reagents are handled.
 - 6) Wear disposable gloves throughout the test procedure. Dispose of gloves in the biohazard waste. Wash hands thoroughly afterward.
 - 7) Wipe spills promptly with 1% sodium hypochlorite solution (1 to 5 dilution of liquid household bleach).
Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite solutions

(bleach) to avoid formation of poison gas. Contaminated materials should be disposed of in the biohazard waste.

- 8) Dispose of all specimens and materials used in the **MeDiPro anti-EBV IgA ELISA** procedure in the biohazard waste. The recommended method of disposal is to disinfect by autoclaving for 1 hour at 121°C followed by incineration. Mix liquid wastes with an equal volume of 5% sodium hypochloride (liquid household bleach) and let stand for 60 minutes before disposal.
- 9) The Controls and 20x concentrated Washing Solution contain 0.05% Thimerosal which can be absorbed through the skin and is a sensitizing agent, please handle carefully.
- 10) The TMB Substrate Solution contains tetramethylbenzidine, hydrogen peroxide and dimethylsulfoxide, it should be disposed appropriately.
- 11) The Stop Solution contains hydrochloric acid. Wear disposable gloves and protective glasses when using and disposing of this reagent.

2. Performance considerations

- 1) Do not use kit components beyond the expired date. **Do not mix components from different lot numbers** except Substrate (TMB) solution, Stop Solution (1N HCl) and 20x Washing Solution. Serum Diluents supplied with **MeDiPro anti-EBV IgA ELISA** kits can be used only in the kits. Do not mix with components from other manufacturers.
- 2) Avoid microbial contamination of reagents. Microbial contamination may interfere with the sensitivity of the assay. When not in use, return all reagents and kit components to refrigerated storage (2 to 8° C).
- 3) Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results. Do not interchange vial or bottle caps and stoppers; this will lead to cross contamination of the reagents. **Do not pour reagents back into vials as reagent contamination may occur.**
- 4) Shield Substrate (TMB) solution from light. Aliquot only the volume of reagents that is needed. Please do not use Substrate (TMB) when blue color occurred. Do not return used Substrate to the bottles.
- 5) To avoid substances which may interfere with the assay, use reagent grade quality water (deionized water that is bacteria free) to dilute the 20x concentrated Washing Solution.

STORAGE INSTRUCTIONS:

1. Store **MeDiPro anti-EBV IgA ELISA** kits and/or sealed individual reagents at 2 to 8°C.
2. Opened, unused microplate strips must be stored at 2 to 8°C in their original bag with the desiccant provided.
3. Store diluted 1x Wash Solution at room temperature (21 to 25°C) for up to 2 weeks.
4. Avoid storing reagents and specimens in auto-defrost refrigerators.

PREPARATION OF REAGENTS:

1. All the reagents and serum samples should be brought to **room temperature (21-25°C)** and mix thoroughly before assay. Do not use reagents beyond the stated expiration date marked on the package label.
2. Washing Solution: dilute 1 volume of 20x concentrated Washing Solution with 19 volume of reagent grade water. If the kit is used for multiple times, please prepare appropriate volume for each time.
3. **All serum samples should be vortexed before use.**
4. Dilution of serum samples: dilute serum samples with Serum Diluents provided in this kit as follows:

10 µl of serum + 1 ml of Serum Diluents

For manual dilutions it is suggested to dispense the Serum Diluents into the test tube first and then add the patient serum. **Controls don't need to be diluted.**

PROCEDURE:

1. Take enough strips and set on strip holder.
2. Add 100 µl of Serum Diluents (blank), Negative Control and Positive Controls (A, B, C) and diluted serum samples to the individual well. Duplication is recommended for each control and blank.
3. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **60 min**.
4. Aspirate or shake out liquid from all wells. Add 280-300 µl of diluted 1x Washing Solution. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure 2 times (for a total of 3 washes). Repeat the wash procedure 4 times (for a total of 5 washes) on automated equipment.

****IMPORTANT NOTE:** Regarding steps 4 and 7 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (280-300 µl) is recommended. A total of 5 washes may be necessary with automated equipment. Complete removal of the washing solution after the last wash is critical for the accurate performance of the test. Also, visually inspect to ensure that no bubbles are remaining in the wells.

5. Add 100 µl of Conjugate to each well.
6. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **30 min**.
7. Aspirate or shake out liquid from all wells. Wash with diluted 1x Washing Solution 3 times. (5 times for semi-automated or automated equipment)
8. Add 100 µl of Substrate (TMB) to each well.
9. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **10 min**.
10. Add 100 µl of Stop Solution (1N HCl) to each well to stop the reaction. Tap the plate gently along the outsides to mix contents of the wells thoroughly.
11. Determine absorbance by reading assay plate at 450 nm using 650 nm as reference. The plate may be held up to 30 min after addition of the Stop Solution before reading.

INTERNAL QUALITY CONTROL:

Results of an assay run are valid if the following criteria for the Controls are met:

1. Average blank (buffer only) well OD_{450nm-650nm} value < 0.05
2. Average OD_{450nm-650nm} value of control sera:
Positive Control A: > 1.50
Positive Control C: > 0.30
Negative Control: < 0.2

If any value is out of the range of above criteria, the assay need repeat.

RESULTS:

1. Calculate the EU:

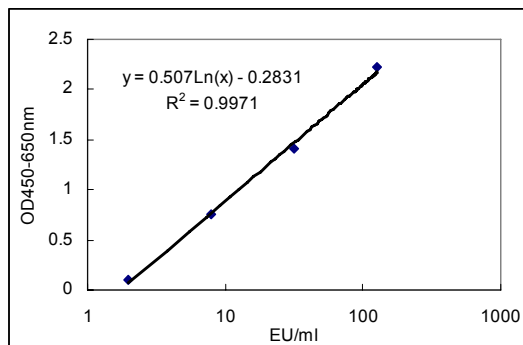
Draw a standard curve on a semilog paper or computer analysis software (Microsoft Excel, Lotus123, etc.) by using OD_{450nm-650nm} of positive control (A, B, C) and negative control and corresponding EU/ml (128, 32, 8, 2

EU/ml). The EU/ml of each positive serum sample can be derived from the equation:

$$Y (OD_{450nm-650nm}) = a \times \ln(X) + b.$$

For example:

	EU/ml	OD _{450nm-650nm}
Positive Control A	128	2.223
Positive Control B	32	1.410
Positive Control C	8	0.762
Negative Control	2	0.096



2. Cut-off value (C.O.V.):

C.O.V. = Calculated OD value of 8 EU/ml

For example:

Calculated OD value of 8 EU/ml = 0.771

	OD Value
C.O.V.-10%	0.694
C.O.V. (8 EU/ml)	0.771
C.O.V.+10%	0.848

The concentration (EU/ml) can be calculated by the equation of standard curve. If the OD_{450nm-650nm} value of serum sample is higher than 0.848, the result of this sample is positive, for confirmation of NPC, further examination on clinical symptoms is necessary; while the OD_{450nm-650nm} value is lower than 0.694, the sample is negative. If OD_{450nm-650nm} value of serum sample is between 0.694~0.848 (C.O.V.±10%), repeat testing is recommended.

LIMITATIONS OF USE:

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. This product is specifically designs for differentiating nasopharyngeal carcinoma patients from normal population. The performance characteristics have not been established for patients with Burkitt's lymphoma, other EBV associated lymphadenopathies, and other EBV associated diseases other than EBV related mononucleosis.
3. The results of ELISA immunoassays performed on serum from immunosupressed patients must be interpreted with caution.
4. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
5. Results of this test should be interpreted by the physician in the light of other clinical findings and diagnostic procedures.
6. Heterotypic (false positive) IgA responses to EBV may occur in patients infected with CMV and also in patients

infected with HSV-1.

7. Specific IgG may compete with the IgA for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction.
8. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
9. Results from children should be reviewed with caution.
10. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.
11. Kit procedures or practices outside those in this package insert may yield questionable results.
12. The performance characteristics have not been established for any matrices other than sera.
13. The performance characteristics for this assay have not been established for pediatric specimens.

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