



User's Manual

Human Adenovirus IgA (ADV-IgA) ELISA Kit



DEIA1383



96T



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

This immunoassay kit allows for the qualitative determination of ADV-IgA in human serum or plasma.

Principles of Testing

This kit was based on indirect ELISA. ADV-Ag was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP- Conjugates, if there were any ADV-IgA in the samples, it would form a ADV-Ag- ADV-IgA- HRP- Conjugates complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Reagents And Materials Provided

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	12 × 8	2-8℃/-20℃
ADV-IgA Positive Control	0.5ml×1	2-8℃
ADV-IgA negative Control	0.5ml×1	2-8℃
sample dilution buffer	12ml×1	2-8℃
HRP- Conjugates	12ml×1	2-8℃
TMB substrate A	6ml×1	2-8℃ (shading light)
TMB substrate B	6ml×1	2-8℃ (shading light)
Stop solution	6ml×1	2-8℃
Wash buffer (20X)	25ml×2	2-8℃
Plate Sealer	3pieces	
Product Description	1 copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2-8°C for 6 months

Specimen Collection And Preparation

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000xg for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500xg within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation

Wash Buffer Preparation

Dilute 50mL of Concentrated Wash Buffer into 950 mL of Wash Buffer with deionized or distilled water.

Assay Procedure

Washing Notes

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Repeat this procedure 2 more times for a total of 3 washes.

Automatic: Aspirate all wells, then wash plate 3 times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

1. Label the sample wells, 3 Negative Controls, 1 Positive Controls and 1 blank wells.
2. Add 100µL Negative Controls and Positive Controls to each wells(except blank well).
3. Add 100µL sample dilution buffer to sample wells and then add 10µL sample serum or plasma. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 50 min.
4. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1-2 minute each time.
5. Add 100 µL HRP- Conjugates to each well, except blank well.
6. Seal the plate with a cover and incubate at 37°C for 30 min.
7. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1-2 minute each time.
8. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.
9. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Quality Control

1. The blank well (only adding TMB and Stop solution) should not be greater than 0.08.
2. The positive control (PC) A value was greater than 0.80.
3. The negative mean A value was less than 0.08.

Calculation

Calculation of the Cut-off Value: **Cut-off Value = NCx+0.1**

NCx: Mean Absorbance of Negative Control(When the negative mean A value is less than 0.05, it is calculated as 0.05. When the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.)

Interpretation Of Results

1. Sample with absorbance values \leq Cut-off Value are NON-REACTIVE and are considered NEGATIVE for ADV-IgA.
2. Sample with absorbance values $>$ Cut-off Value are considered POSITIVE for ADV-IgA.

Precautions

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 mins.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive.

5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.