Product Manual

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Human Anti-Immunoglobulin A Antibody (Anti-IgA) ELISA Kit

Catalog No.: abx052587

Size: 96T

Range: 20 µg/ml - 320 µg/ml

Sensitivity: 1.0 µg/ml

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of Anti-IgA in Human Serum, Plasma and other biological fluids.

Introduction: Immunoglobulin A (IgA, also referred to as sIgA in its secretory form) is an antibody that plays a crucial role in the immune function of mucous membranes. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibody combined. In absolute terms, between three and five grams are secreted into the intestinal lumen each day. This represents up to 15% of total immunoglobulins produced throughout the body.

Principle of the Assay

A 96 well plate has been pre-coated with the target antigen. An HRP conjugated antibody is used as detection antibody. The standards, test samples and HRP conjugate reagent are added to the wells and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the Anti-IgA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Anti-IgA can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Human Anti-IgA Standard: 0.3 ml x 6 Tubes
3. Wash buffer (20X): 25 ml. Dilution: 1:20
4. Sample diluent buffer: 6 ml
5. HRP Conjugate Reagent (RTU): 10 ml
6. Stop solution: 6 ml
7. TMB substrate A: 6 ml
8. TMB substrate B: 6 ml
9. Plate sealer: 2
10. Hermetic bag: 1

Material required, not provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 x g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as the anticoagulant. Centrifuge for 15 min at 1000 × g within 30 min of collection. Analyze immediately or aliquot and store frozen at - 20 °C or -80°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» The QC data has been produced using a 1:5 dilution with the provided Sample diluent buffer. If the sample concentration is unknown, we would recommend a starting point of 1:5 dilution with the provided Sample diluent buffer.

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 20 ml of concentrated wash buffer into 380 ml of distilled water).

3. Standard

6 tubes of standard are included in each kit. Please refer to the table below for the concentrations of standard in each tube.

Standard tube no.	S0	S1	S2	S3	S4	S5
Concentration (µg/ml)	0	20	40	80	160	320

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively and record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls.
2. Aliquot 50 µl of the diluted standards (320 µg/ml, 160 µg/ml, 80 µg/ml, 40 µg/ml, 20 µg/ml) into the standard wells.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
4. Seal the plate with a cover and incubate at 37°C for 30 min.

5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Aliquot 100 µl of HRP Conjugate Reagent into each well (except control well).
7. Seal the plate with a cover and incubate at 37°C for 60 min.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
9. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
10. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (relative O.D.450) = (O.D.450 of each well) – (O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Human Anti-IgA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1.Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.

2.If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3.Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

4.Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.

5.Ensure that the plate is properly sealed or covered during incubation steps.

6.Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

7.To avoid cross contamination do not reuse pipette tips and tubes.

8.The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial..

D. Typical Data & Standard Curve

Results of a typical standard run of a Human Anti-IgA ELISA Kit is shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

Concentration µg/ml	0	20	40	80	160	320
OD450	0.045	0.133	0.228	0.455	0.774	1.602