ZeptoMetrix®

Human IgA ELISA

Catalog Number:
0801197

INTENDED USE

The IMMUNO-TEK Human IgA ELISA Kit is a rapid, easy to use **e**nzyme-**l**inked **i**mmuno**s**orbent **a**ssay (ELISA) designed for the measurement of human IgA in serum, plasma, cell culture supernatants or other biological fluids. The assay contains ready-to-use reagents and takes less than two hours to perform.

The IMMUNO-TEK Human IgA ELISA Kit is for Research Purposes Only.

PRINCIPLE OF THE TEST

Microwells coated with polyclonal antibodies to human IgA form the capture phase of the assay. Captured human IgA then reacts with detector antibody which is a polyclonal anti-human IgA conjugated with horseradish peroxidase. Enzyme activity in the wells is then quantified using tetramethyl benzidine as a substrate.

REAGENTS

Materials Supplied:

- **Microplate, (1x96 well): Strips coated with purified goat anti-human IgA**
- **Detector Antibody (12 mL): Contains conjugated goat anti-human IgA peroxidase**
- **Human IgA Standard (7 mL): Contains human IgA and assay diluent**
- **Assay Diluent (100 mL): Contains PBS, Triton X-100® and 2-chloroacetamide**
- **Plate Wash Buffer (125 mL): Contains PBS, Tween 20® and 2-chloroacetamide**
- **Substrate (12 mL): Contains Tetramethyl Benzidine (TMB)**
- **Stop Solution (12 mL): Proprietary formulation**
- **Microtiter Plate Sealers (1 pk): 10 sealers per pack**
- **Plastic Bag (1 bag): For storage of unused microtiter plate strips**

® Triton X-100 is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.
Tween 20 is a registered trademark of Imperial Chemical Industries.

Materials Required but not Supplied:

- Disposable gloves
- Test tubes and racks for preparing specimen and IgA standard dilutions
- Validated adjustable micropipettes, single and multi-channel
- Distilled or deionized water
- Graduated cylinders and assorted beakers
- Validated microtiter plate reader
- Automatic microtiter plate washer or manual vacuum aspiration equipment
- Timer

STORAGE

Store all kit reagents at 2-8°C. Do not freeze. Unused microplate strips should be kept in a sealed bag with desiccant to minimize exposure to moisture. All reagents stored and handled properly are stable at least until the expiration date printed on the kit box label.

PRECAUTIONS

FOR RESEARCH USE ONLY. Not For *in vitro* Diagnostic Use.

- Prior to performing the assay, carefully read all instructions.
- Use universal precautions when handling kit components and test specimens.*
- To avoid cross-contamination, use separate pipette tips for each specimen.
- When testing potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
- Stop Solution contains hydrochloric acid, which may cause severe burns. In case of contact with eyes or skin, rinse immediately with water and seek medical assistance. Wear protective clothing and eyewear.

*MMWR, June 24, 1988, Vol. 37, No. 24, pp. 377-382, 387-388

PREPARATION OF REAGENTS

Plate Wash Buffer:

Dilute 10X Plate Wash Buffer 1:10 in distilled or deionized water prior to use. Mix thoroughly. Prepared 1X Plate Wash Buffer can be stored at 2-8°C for up to one week. Additional 10X Plate Wash Buffer (ZEPTOMETRIX Catalog #: 0801060) may be ordered if needed.

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Human IgA Standard Curve

Label 6 test tubes as shown below. The Human IgA Standard is provided at 125 ng/mL. This should be diluted in Assay Diluent as follows to prepare a standard curve.

Tube Number	Concentration of Human IgA	Volume of Human IgA Standard	Volume of Assay Diluent
1	125 ng/mL	1000 µL	0 µL
2	62.5 ng/mL	500 µL of #1	500 µL
3	31.25 ng/mL	500 µL of #2	500 µL
4	15.6 ng/mL	500 µL of #3	500 µL
5	7.8 ng/mL	500 µL of #4	500 µL
6	0 ng/mL	0 µL	500 µL

SPECIMEN DILUTIONS

Serum and Plasma:

Human serum and plasma samples typically contain 0.6-4.0 mg/mL of IgA. Therefore, we recommend preparing a 1:40,000 dilution of the sample in Assay Diluent for initial testing.

After initial testing, it may be necessary to adjust the concentration of the antibody solution to be tested in order to obtain a concentration between 125 ng/mL and 7.8 ng/mL for accurate quantification.

TEST PROCEDURE

Allow all reagents to reach room temperature before use. Label test tubes to be used for the preparation of standards and specimens. If the entire 96-well plate will not be used, remove surplus strips from the plate frame and place into the resealable Plastic Bag with desiccant. Seal bag and store at 2-8°C.

Step 1: Label each strip on its end tab to ensure identity should the strips become detached from the plate frame during the assay.

Step 2: Designate one well on the plate and leave empty. This well will serve as a substrate blank.

Step 3: Pipette 200 µL of standards #1-6 into duplicate wells.

Step 4: Pipette 200 µL of each specimen into duplicate wells.

Step 5: Cover the microplate with a plate sealer and incubate the plate for 30 minutes at room temperature.

Step 6: Aspirate the contents of each well and wash the wells 4 times with 1X Plate Wash Buffer. To wash, fill the wells with 300 µL of 1X plate wash buffer and aspirate. Perform 4 fill/aspirate cycles. After the final wash cycle, thoroughly blot the plate by carefully striking the plate on a pad of absorbent paper towels. Continue until no visible droplets of Plate Wash Buffer are observed

Step 7: Pipette 100 µL of Detector Antibody into each standard and specimen well. **Do not add Detector Antibody to the substrate blank well.**

Step 8: Cover the plate with a plate sealer and incubate for 30 minutes at room temperature.

Step 9: Wash the plate 4 times with Plate Wash Buffer as described in **Step 6.**

Step 10: Pipette 100 µL of Substrate into each well **including** the substrate blank well.

Step 11: Incubate the plate for 30 minutes at room temperature. A blue color will develop in wells containing human IgA.

Step 12: Pipette 100 µL of Stop Solution into each well. A color change from blue to yellow will occur.

Step 13: Within 15 minutes, read the optical density of each well at **450 nm** using a microtiter plate reader.

CALCULATION AND INTERPRETATION OF RESULTS

1. Calculate the mean optical density (OD) values for each set of standards and diluted specimen samples.
2. Using linear graph paper or graphing software, plot the mean OD values of each standard on the Y-axis versus the corresponding concentration of standards on the X-axis.
3. Draw the best fit curve through these points to construct a standard curve. In most cases a linear regression plot will be sufficient but for the most accurate results use a point-to-point or 4-parameter logistic regression.
4. Determine the IgA concentration of each diluted specimen sample by interpolation from the standard curve.
5. Multiply by the dilution factor used to dilute each specimen sample to determine the IgA concentration of the original specimen sample.

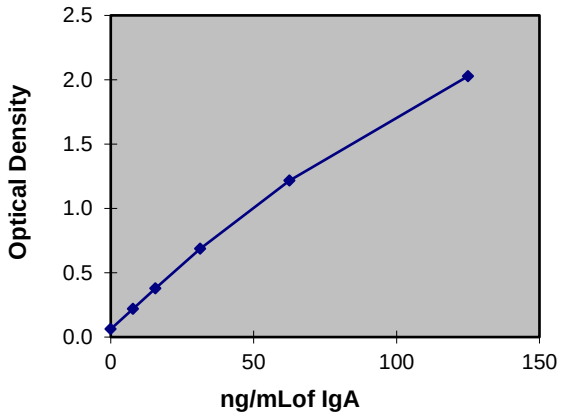
Test Validity:

- For the test to be valid, the mean optical density of the 0 ng/mL standard and the substrate blank must be below 0.200.
- The mean optical density of the 125 ng/ml must be above 1.000.
- It is recommended that specimen samples be re-assayed with a greater or lesser dilution when the mean optical density value does not fall within the standard curve.

EXPECTED RESULTS

Below is an example of a standard curve and should not be used to calculate actual samples. Variations may be observed from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

Human IgA Standard Concentration	Optical Density at 450 nm
125 ng/mL	2.028
62.5 ng/mL	1.216
31.25 ng/mL	0.687
15.6 ng/mL	0.377
7.8 ng/mL	0.220
0 ng/mL	0.062



PROCEDURAL FLOW CHART

PREPARE REAGENT DILUTIONS
↓
PIPETTE SPECIMENS AND STANDARDS
↓
INCUBATE 30 MINUTES AT ROOM TEMPERATURE
↓
WASH PLATE
↓
PIPETTE DETECTOR ANTIBODY
↓
INCUBATE 30 MINUTES AT ROOM TEMPERATURE
↓
WASH PLATE
↓
PIPETTE SUBSTRATE SOLUTION
↓
INCUBATE 30 MINUTES AT ROOM TEMPERATURE
↓
ADD STOP SOLUTION AND READ AT 450 NM

For Research Use Only NOT for *in vitro* Diagnostic Use

REF	Catalog Number		Temperature Limitation
LOT	Batch Code		Expiration Date
RUO	For Research Use Only		Biological Risk

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