GENTAUR

DATA SHEET

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IMMUNOtek

Quantitative
Human IgA ELISA

ZMC Catalog #: 0801197

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

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, hybridoma cell supernatants, ascites or other biological fluids. The kit is especially useful in monitoring the production and purification of human monoclonal antibodies. 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.

Storage:

Store all kit reagents at 2-8 ° C. Do not freeze.

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
- Validated incubator capable of maintaining 37 ± 1°C
- Graduated cylinders and assorted beakers
- Validated microtiter plate reader
- Automatic microtiter plate washer or manual vacuum aspiration equipment
- Timer

PRECAUTIONS

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- 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 (ZMC Catalog #: 0801060) may be ordered if needed.

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.

Hybridoma Supernatants:

Hybridoma supernatants from stationary cell cultures will typically contain between 1 µg/ml and 30 µg/ml of monoclonal antibody. Therefore, we recommend preparing a 1:250 dilution of cell culture supernatants in Assay Diluent for initial testing.

When using cell culture supernatants from bioreactors, a further dilution may be necessary since many bioreactors are capable of producing much higher concentrations of monoclonal antibodies than standard stationary cell cultures. Refer to the technical literature provided with the bioreactor to determine a dilution that will yield a monoclonal antibody concentration between 125 ng/ml and 7.8 ng/ml.

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.

Ascites:

Ascites fluid will typically contain between 1 mg/ml and 10 mg/ml of monoclonal antibody. Because of this, we recommend preparing a 1:100,000 dilution of ascites 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 37°C.

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 37° C.

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.

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.

CALCULATION AND INTERPRETATION OF RESULTS

Using linear graph paper or a computer program, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis.

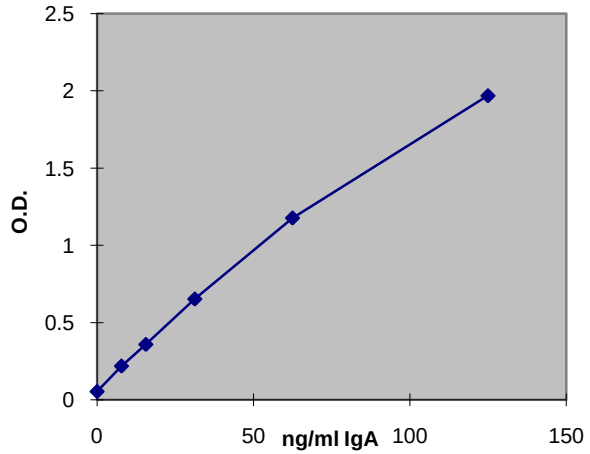
The concentration of human IgA in each diluted specimen may then be determined manually using a ruler to extrapolate, by linear regression using a computer program or pocket calculator with a linear regression function, or by point to point calculation again using a computer or calculator.

Correct the diluted specimen values by the dilution factor used to obtain the final concentration of human IgA in the original specimen.

Typical Standard Curve

Below is an example of a typical standard curve. Variations will occur from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

Human IgA Standard Concentration	Optical Density at 450 nm
125 ng/ml	1.969
62.5 ng/ml	1.177
31.25 ng/ml	0.652
15.6 ng/ml	0.358
7.8 ng/ml	0.218
0 ng/ml	0.065
Substrate Blank	0.054



PROCEDURAL FLOW CHART

PREPARE REAGENT DILUTIONS

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PIPETTE SPECIMENS AND STANDARDS

↓

INCUBATE 30 MINUTES AT 37° ± 1°C

↓

WASH PLATE

↓

PIPETTE DETECTOR ANTIBODY

↓

INCUBATE 30 MINUTES AT 37° ± 1°C

↓

WASH PLATE

↓

PIPETTE SUBSTRATE SOLUTION

↓

INCUBATE 30 MINUTES AT ROOM TEMPERATURE

↓

ADD STOP SOLUTION AND READ AT 450 NM

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium