IgA Total ELISA

Catalog Number: IGA31-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.

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**Intended Use:**

The Eagle Biosciences IgA ELISA assay kit is intended for the quantitative determination of total IgA in serum or plasma by enzyme linked immunoassay (ELISA). The IgA ELISA assay kit is for research use only and not to be used in diagnostic procedures.

**Assay Background:**

Immunoglobulins have important roles in the humoral immune response (HIR), and they consist of 5 major isotypes: immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin D (IgD), and immunoglobulin E (IgE). The most abundant isotype of immunoglobulins in the blood is IgG which comprises 73% of total immunoglobulin and has a molecular weight of 150 kd. IgA is present in plasma and external secretions and is expressed on the B-cell membrane.

It is possible to distinguish forms of IgA based upon their location - serum IgA vs. secretory IgA. To measure secretory IgA (sIgA), please utilize the Eagle Biosciences sIgA ELISA (SGA35-K01). The high prevalence of IgA in mucosal areas is a result of cooperation between plasma cells that produce polymeric IgA (pIgA), and mucosal epithelial cells that express an immunoglobulin receptor called the polymeric Ig receptor (pIgR). pIgA is released from the nearby activated plasma cells and binds to pIgR. This results in transportation of IgA across mucosal epithelial cells and its cleavage from pIgR for release into external secretions.

In the blood, IgA interacts with an Fc receptor called FcαRI (or CD89), which is expressed on immune effector cells, to initiate inflammatory reactions. Ligation of FcαRI by IgA containing immune complexes causes antibody-dependent cell-mediated cytotoxicity (ADCC), degranulation of eosinophils and basophils, phagocytosis by monocytes, macrophages, neutrophils and eosinophils, and triggering of respiratory burst activity by polymorphonuclear leukocytes.

**Principle of Procedure:**

The Total IgA ELISA assay kit determines total IgA according to the “sandwich” principle. IgA in samples and standards binds to antibodies which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The IgA concentration can be calculated from the standard curve.
Materials Provided:

The expiration date for the IgA Total ELISA assay kit and each component is stated on the label(s). Store all components at 2-8°C with the exception of the IgA standard, which should be stored at -20°C.

- Anti-Human IgA coated microwell strips 12x8 with plastic frame
- HRP conjugated goat anti-human IgA -12mL
- IgA standard (pre-diluted 1:10,000)
- TMB/peroxide substrate color developer –12mL
- IgA specimen diluent (Specimen Diluent Green II) -60mL
- Sulfuric acid termination reagent (0.5N) –12mL
- 15 X Wash buffer concentrate – 60mL

Reagent and Sample Preparation:

- Dilute the 15X wash buffer provided 1:15 using one part wash buffer concentrate and 14 parts reagent grade water.
- Dilute each serum or plasma specimen to be tested 1:10,000 with the IgA specimen diluent provided.
- Prepare serial two fold dilutions of the human IgA standard: Neat, 1:2, 1:4, 1:8 etc. with the specimen diluent provided. Use the specimen diluent alone as the blank control well.

Assay Procedure:

Allow each reagent of the Total IgA ELISA assay kit to reach room temperature before use.

1. Add 100uL of diluted specimen or standard to each microwell
2. Incubate at room temperature for 60 minutes
3. Decant and wash each microwell four times with wash buffer (dilute buffer 1:15 with reagent grade water)
4. Add 100uL of HRP conjugated goat anti-human IgA to each well
5. Incubate at room temperature for 60 minutes
6. Decant and wash as in step 3
7. Add 100uL of TMB/peroxide substrate and incubate at room temperature for 30 minutes
8. Terminate the reaction with 100uL of 0.5N sulfuric acid

Calculations:

For calculating the results we recommend using the 4-parameter algorithm. First, zero the microwell reader at 450 nm using the blank control well. If this algorithm is not available a "point to point" or a "spline" function can be used.

Manual processing of results: Correct each absorbance value by subtracting the background absorbance (blank). Estimate the mean value for each duplicate. Construct a standard curve by plotting the mean absorbance value for each standard (y-axis) against the corresponding concentration (x-axis) on semi-log graph paper and read the concentration of unknowns off the curve.

Typical Standard Curve:

The curve given above is only for demonstration. It must not be used for calculation of your samples.
Dynamic Range:

The dynamic range of the Total IgA ELISA assay kit is 0.031 µg/mL - 2µg/mL.

Reproducibility:

The Total IgA ELISA assay kit was found to have a reproducibility with a C.V. 6% - 10% depending on region of the standard curve.

Warranty Information

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.