# IgA ELISA Kit

IgA ELISA Kit is an Enzyme Immunoassay kit for the quantification of IgA in saliva.

Catalog number: ARG83087

Package: 96 wells

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**MANUFACTURED BY:**

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## INTRODUCTION

Immunoglobulin A (IgA, also referred to as sIgA (secretory IgA)) 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.

IgA has two subclasses (IgA1 and IgA2) and can be produced as a monomeric as well as a dimeric form. The IgA dimeric form is the most prevalent and is also called secretory IgA (sIgA). sIgA is the main immunoglobulin found in mucous secretions, including tears, saliva, sweat, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions. sIgA can also inhibit inflammatory effects of other immunoglobulins. IgA is a poor activator of the complement system, and opsonises only weakly.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IgA has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IgA present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for IgA is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgA bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of IgA in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 100X Antibody Conjugate concentrate at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody Coated Microplate | 8 x 12 strips | 4°C. Unused strips should be sealed tightly in the air-tight pouch. |
| Standard A-E (0, 6.9, 62, 132, 400 ng/ml) | 5 x 1 ml/vial | 4°C |
| Control | 1 ml | 4°C |
| 5X Assay Buffer | 40 ml | 4°C |
| 20X HRP-Streptavidin Solution | 1 vial (120 µL) | 4°C |
| 50X Wash Buffer | 20 ml | 4°C |
| TMB Substrate | 15 ml (Ready to use) | 4°C (Protect from light) |
| Stop Solution | 15 ml (Ready to use) | 4°C |
| Plate sealer | 3 strips | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store 100X Antibody Conjugate concentrate at ≤ -20°C. Store other component at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Saliva- Advised to use centrifuge glass tube for sample collection. Let the saliva flow down through the straw into the centrifuge tube; then centrifuge at 3000 rpm per 15 minutes.

## REAGENT PREPARATION

- 1X Assay Buffer: 20 minutes before use, dilute 5X Assay Buffer with distilled water to yield 1X Assay Buffer. Keep diluted Assay Buffer at 4°C before use.
- 1X HRP-Streptavidin Solution: 20 minutes before use, dilute 20X HRP-Streptavidin Solution with 1X Assay Buffer to yield 1X HRP-Streptavidin Solution.
- 1X Wash Buffer: Dilute 50X Wash Buffer with distilled water to yield 1X Wash Buffer.

  The diluted 1X Wash Buffer is stable for 4 weeks at 2°C to 8°C.
- Sample: Diluent saliva with 1X Assay Buffer in 1:20 dilution, mix well and sit sample for at least 5 min for first dilution. Then diluent the first dilution with 1X Assay Buffer in 1:50 dilution. The final dilution ratio should be 1:1000. (It is recommended to do pre-test to determine the suitable dilution factor).

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

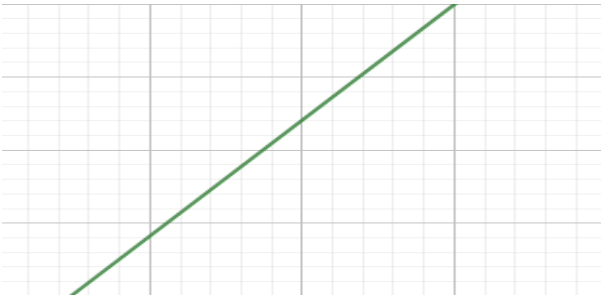
1. Add **25µL** of **Diluted Samples, Control** or **Standard** into respective wells.
2. Add **100µL** of **1X HRP-Streptavidin Solution** into each wells.
3. Cover the plate and incubate for **1 hour** at **room temperature**.
4. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of **TMB Substrate** to each well.
6. Cover the plate and incubate for **15 minutes** at **room temperature**.
7. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
8. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 30 minutes after adding the stop solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the IgA ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



## QUALITY ASSURANCE

### Sensitivity

1.7 µg/ml

### Assay Range

6.9 – 400 µg/ml

### Cross-Reactivity

Not reacts with IgG and IgM.