Human secretory IgA ELISA Kit

Enzyme Immunoassay for the quantitative determination of human secretory IgA in serum, plasma, urine, saliva, breast milk, nasal wash, vaginal secret and bronchoalveolar fluid.

Catalog number: ARG80866

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Secretory IgA (sIgA) is the main immunoglobulin present on mucosal surfaces. Ca. 90% of sIgA is produced locally and does not penetrate into blood circulation. sIgA is considerably different from serum IgA, as this complex protein consists of 3 completely different molecules. Two or four molecules of immunoglobulin A with molecular weight 160 kDa are joined by J-chain (16 kDa) and attached to the secretory component (80kDa); the formation of this complex occurs during transepithelial transport of polymeric IgA. sIgA plays a pivotal role in local immunity by blocking bacterial and viral adhesion and invasion through epithelial tissues. Determination of sIgA concentration allows to evaluate the local immunity status in stomatology, ophthalmology, respiratory diseases, gastroenterology, gynaecology. The sIgA in saliva can be also used as noninvasive mass screening for selective IgA deficiency. Elevation of sIgA in serum is occasionally observed in so autoimmune diseases and several tumours.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Secretory IgA has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Secretory IgA present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for IgA alpha chain is added to each well and incubate. 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 Secretory 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 450 nm ±2 nm. The concentration of Secretory IgA in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-anti-IgA alpha chain antibody Conjugate	14 ml (ready to use)	4°C
Standards 1-6 (0, 2, 20, 40, 100, 400 μg/ml)	6 X 1 ml (ready to use)	4°C or -20°C
Control (Acc. range: 40-70 μg/ml	1 ml (ready to use)	4°C or -20°C
Assay Buffer (red)	22 ml (ready to use)	4°C
Sample diluent (blue)	100 ml (ready to use)	4°C
26 X Wash buffer	22 ml (ready to use)	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C
Plate sealing tape	2 pcs	4°C

Note: Opened Standards and control should be aliquoted and stored at ≤ -20 °C for up to 2 months. Avoid repeated freeze-thaw cycles, and only one freezing/thawing cycle is allowed.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 20-25°C) before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent

contamination may occur.

- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using ACD or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Saliva- Do not eat and avoid vigorous physical activity 60 minutes before sample collection. Document consumption of alcohol, caffeine, nicotine and prescription / over-the-counter medications within the prior 12 hours. Document the presence of oral diseases or injury. Before collecting saliva, rinse mouth with water to remove food residue and wait at least 10 min after rising to avoid sample dilution. Before assaying, saliva samples should be frozen at -20°C overnight, then thawed and centrifuge at 1000xg for 10 min to remove particulates. Undiluted samples could be stored at 4°C for up to 5 days. For

long term storage, aliquot and store samples at ≤ -20 °C for up to 1 month. Avoid repeated freeze-thaw cycles.

Urine- Use a sterile container to collect urine samples. Centrifuge at 1000xg for 10 min at 4°C to remove particulates. Collect the supernatant, assay immediately or aliquot and store samples at ≤ -20°C or -80°C for up to 1 month. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional particulates that may appear after storage.

Other samples (bronchoalveolar fluid, nasal wash, vaginal secret and breast milk) - Centrifuge to precipitate particulates and force mucus to the bottom of the tube. Collect the supernatant, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 26X Wash buffer into distilled water to yield 1X Wash buffer. E.g. 22ml of 26X Wash buffer + 550ml of distilled water. Diluted 1X wash buffer can be stored at 4°C for up to 1 month.
- Sample dilution:
  - A. Step 1. Before Assay Pre-dilute samples by Sample diluent (blue) as the dilution instruction as below:
  - Blood serum, plasma, saliva, bronchoalveolar fluid, nasal wash and vaginal secret: Dilute 1:100 with Sample diluent (blue). (e.g. 5 µl sample + 500 µl Sample diluent (blue)
  - Breast milk: Dilute 1:500 with Sample diluent (blue). (e.g. 5 µl sample + 2500 µl Sample diluent (blue)
  - Urine: Dilute 1:50 with Sample diluent (blue). (e.g. 10 µl sample + 500 µl Sample diluent (blue)
  - B. Step 2. Add samples and Assay buffer (red) in the sample wells: Add Assay Buffer (red) as dilution instruction as below and then add the diluted samples from step 1 into the sample wells on the antibody-coated microplate.
  - saliva : 10 µl diluted sample + 190 µl Assay Buffer (red);
  - nasal wash: 20 µl diluted sample + 80 µl Assay Buffer (red);
  - vaginal secret: 10 µl diluted sample + 90 µl Assay Buffer (red);
  - breast milk: 5 µl diluted sample + 195 µl Assay Buffer (red)
  - Serum, plasma, bronchoalveolar fluid or Urine: 100 µl diluted sample + 0 µl Assay Buffer (red)

C. Dilution Summary and dilution factors:

Sample type	Step 1 Before assay		Step2 Add into wells		Dilution factor
	Sample diluent (blue) (µl)	Sample (µl)	Assay Buffer (red) (µl)	Diluted samples from step1 (µl)	
Serum	500	5	0	100	0.05
plasma	500	5	0	100	0.05
saliva	500	5	190	10	1.0
urine	500	10	0	100	0.025
bronchoalveolar fluid	500	5	0	100	0.05
nasal wash	500	5	80	20	0.25
vaginal secret	500	5	90	10	0.5
breast milk	2500	5	195	5	10
Control	-	-	-	-	-
Standards	-	-	-	-	-

Note: Do not dilute control and Standards

D. If the initial assay found samples contain IgA higher than the highest standard, the samples can be further diluted with Sample diluent buffer (blue) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation. It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge. Standards, samples and controls should be assayed in duplicates.

1. **Dilute samples** with **Sample buffer (blue)** as the dilution instruction in REAGENT PREPARATION section-Sample dilution-A or C. summary table.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
3. Add **Assay Buffer (red)** into sample wells and then add **diluted samples from step 1** into sample wells as the dilution instruction in REAGENT PREPARATION section-Sample dilution-B or C. summary table.
4. Add **100 µl Standards and controls** in duplicate into the appropriate wells. Control and Standards are ready to use, and DO NOT dilute control and Standards.
5. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5-7 seconds. Cover the wells by plate adhesive tape and **incubate for 90 minutes at 37°C**.
6. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X wash buffer (250 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 distilled water by aspirating, decanting or blotting against clean

paper towels.

7. Add 100 µl of HRP-antibody conjugate into each well.
8. Cover wells and incubate for 30 minutes at 37°C.
9. Aspirate and wash wells for a total 5 washes as step 6.
10. Add 100 µl of TMB substrate to each well. Incubate for 10-20 minutes at room temperature (18-25°C) in dark.
11. Add 100 µl of Stop Solution to each well.
12. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using 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) or point-to-point curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor and the calculation factors for different samples are as below:

−saliva: 1

−urine: 0.025

−nasal wash: 0.25

−vaginal secret: 0.5

− breast milk: 10

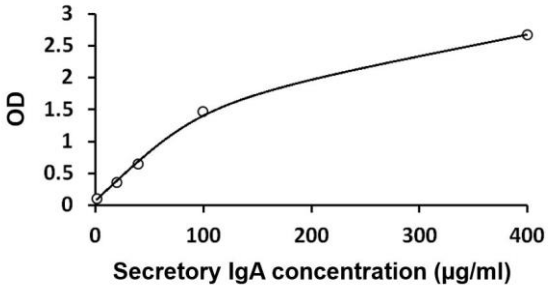
−Serum, plasma or bronchoalveolar fluid: 0.05

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 further 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 data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.6 μg/ml.

Specificity

The following substances were tested for cross reactivity of the assay:

	Cross-reactivity (%)
IgG	< 0.1
IgM	< 0.1
IgE	< 0.1

Linearity

90-110%

Recovery

90-110%