Secretory IgA ELISA

For the quantitative determination of sIgA in human stool

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 31-SECHU-E01

Size: 96 determinations

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26-G Keewaydin Drive, Salem, NH 03079|P: (800) 592-5726|F: (603) 898-6854|ts@alpco.com|www.alpco.com

INTENDED USE

The Secretory IgA ELISA is intended for the quantitative determination of secretory immunoglobulin A (sIgA) in human stool. For Research Use Only. Not for Use in Diagnostic Procedures.

INTRODUCTION

Secretory IgA consists of two immunoglobulin A molecules connected to each other by a J protein (joining) and a secretory component. The secretory component is synthesized by the epithelial cells of the mucous membranes of the gastrointestinal, respiratory, and urogenital tracts, as well as in the salivary, lacrimal, and mammary glands. The plasma cells in the subendothelial space of the mucous membranes secrete a complex of two IgA molecules linked via the J protein. This complex then binds to the secretory component that sits on the surface of the epithelial cell. After binding, the sIgA is transported through the epithelial cell and excreted at the mucosal surface by exocytosis.

The determination of the secretory IgA (sIgA) provides an initial overview of the functional status of the gut-associated immune system (GALT). Here, the secretion capacity and the degree of stimulation of the plasma cells in the submucosa of the intestine are recorded.

PRINCIPLE OF THE ASSAY

The Secretory IgA ELISA test determines human secretory IgA according to the “sandwich” principle. sIgA in samples, standards, and controls binds to antibodies which are coated to the microtiter plate. After a wash step a peroxidase-labeled detection antibody is added. A second wash 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 (against the reference wavelength 620 nm) in a microtiter plate reader. The sIgA concentration can be calculated from the standard curve.

MATERIALS SUPPLIED

31-SECHU-E01			
Component	**Quantity**	**Preparation**	**Storage**
Microtiter plate, coated	1 plate: 12 x 8-well strips	Ready-to-use	2-8˚C
Universal extraction buffer	150 mL	Ready-to-use	2-8˚C
Wash Buffer concentrate, 10X	100 mL	Dilute 1:10	2-8˚C
Standards*	5 vials, 1 mL each	Ready-to-use	2-8˚C
Control 1 and 2*	2 vials, 1 mL each	Ready-to-use	2-8˚C
Conjugate: peroxidase-labeled antibody	15 mL	Ready-to-use	2-8˚C
TMB Substrate: tetramethylbenzidine	15 mL	Ready-to-use	2-8˚C
Stop solution	10 mL	Ready-to-use	2-8˚C
Plate Sealers	2	Ready-to-use	RT

*Please refer to the Certificate of Analysis enclosed with each kit for Standard and Control concentrations.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge, 3000 x g
- Plastic vials
- Stool sample extraction vials (80-EXDEV2-100)
- Vortex mixer
- Various pipettes
- Multichannel or multipipette
- Foil to cover the microtiter plate (substrate step)
- Distilled or deionized water
- ELISA reader with 450 nm filter (620 nm reference filter)
- Microtiter plate shaker (2 mm orbital shaker capable of 400 rpm)
- Absorbent paper (lint-free)
- Timer

PRECAUTIONS

- This assay is for Research Use Only. Not for use in diagnostic procedures.
- Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.
- The test kit reagents contain preservatives to protect against bacterial growth. Avoid contact with the skin and/or mucous membranes.
- The test kit contains components of human origin. Standards and controls contain sIgA from human colostrum. The starting reagent was examined for all parameters required by the FDA. All parameters tested were found negative. As a precautionary measure, all test kit reagents should always be treated as potentially infectious material in accordance with health care accident prevention regulations.
- The substrate TMB (tetramethylbenzidine) is toxic by ingestion and skin contact. In the event of contact with the skin, the affected area must be washed immediately with plenty of water and soap.
- Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. Work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.
- Adherence to the test protocol is essential. ALPCO assumes no liability for any damage caused by unauthorized changes in the test procedure.
- The guidelines for carrying out quality control in testing laboratories must be observed. Appropriate controls must be tested.
- The reagents must not be used after the expiration date.
- Wear disposable gloves when handling samples or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or apply makeup in areas where samples or kit reagents are being handled.
- Samples may contain unknown interfering substances. This can lead to false high or false low results.

REAGENT PREPARATION

Microtiter plate - Assemble the required number of strips in the holder. Allow the plate to reach 20-30°C before use. Unused strips must be stored at 2-8°C in the pouch with desiccant. Please do not dispose of the holder until all strips are used.

Wash buffer - Please note: When storing the wash buffer concentrate at 2-8 °C crystallization may occur. Before dilution, all crystals must be dissolved. Dilute the wash buffer concentrate 1:10 with distilled or deionized water (1 part buffer + 9 parts DI water). The 1X working wash buffer is stable for 14 days at 2-8°C. Dilute only the amount of buffer needed to process the given samples.

All other test reagents are stable at 2-8 °C up to the date of expiry stated on the label, unless otherwise specified.

SAMPLE PREPARATION

Stool samples must be extracted with Universal Extraction Buffer at a ratio of 1:100 (e.g. 10 mg/ml) before testing in the Secretory IgA ELISA. Manual weighing or an approved stool extraction device (80-EXDEV2-100) may be used.

For manual weighing, mix **15 mg** stool with **1.5 ml** universal extraction buffer (or greater amount of stool diluted **1:100** with universal extraction buffer), then vortex until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge for 10 min at 3000 x g.

Dilute the supernatant **1:125** in 1x working wash buffer, (e.g. 8 µl supernatant + 992 µl 1x working wash buffer).

ASSAY PROCEDURE

All reagents and samples should be equilibrated to 20-30°C and mixed well before use.
The position of standards, controls, and samples are noted on a protocol sheet.

1. **Wash step**

   Pick out the pre-assembled microtiter plate with the needed number of strips and wash them 1x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the wash step.

2. **Sample incubation**

   Pipette **100 µl STD, CTRL, and diluted samples** in duplicate in the microtiter plate.

   Cover the strips and incubate by shaking for **60 min** at room temperature (20-30 °C; 400 rpm, 2 mm orbit).

3. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate **gently** on absorbent paper after the last wash step.

4. **Conjugate incubation**

   Pipette **100 µl CONJ** in each microwell.

Cover the strips and incubate with shaking for **60 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit).

5. **Wash step**

   Discard the contents of the microwells and wash 5x with 250 µl 1X working wash buffer per well. Remove residual buffer by tapping the plate on absorbent paper after the last wash step.

6. **Substrate incubation**

   Pipette **100 µl TMB substrate** in each microwell.

   Incubate by shaking for **10-15 min** at room temperature (20-30°C; 400 rpm, 2 mm orbit) **in the dark**.

7. **Stopping the reaction**

   Pipette **50 µl stop solution** in each microwell. Mix well.

8. **Reading**

   Read the absorbance at 450 nm. If available, use 620 nm as reference wavelength. Reading should be done within **5 minutes** after stopping the reaction.

CALCULATION OF RESULTS

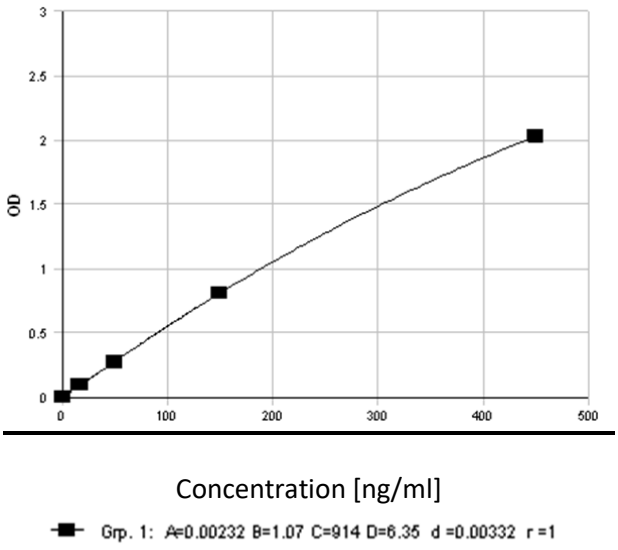
The use of the 4-parameter-Marquardt algorithm is recommended for calculation of results. The Secretory IgA concentration is multiplied by **12.5.**

Dilution 1: 15 mg in 1.5 ml corresponds to a factor of **100** (assumption: 1 g stool = 1 ml)

Dilution 2: Factor **125** (8 µl supernatant + 992 µl 1x working wash buffer)

Calculation: Sample concentration [µg/ml] = obtained conc. [ng/ml] x 100 x 125 / 1000

TYPICAL STANDARD CURVE



The curve at left is for demonstration purposes only. It must not be used for calculation of sample values.

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range of secretory IgA is 208.8 – 5,625 µg/ml.

Sensitivity

Limit of Detection (LOD): 2.2 ng/ml

For the determination of the detection limit, 20 replicates of Standard 0 were measured. The 2x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Limit of Quantification (LOQ): 5.7 ng/ml

For the determination, 20 replicates of Standard 0 were measured. The 2x standard deviation was added to the mean value of the optical density. The respective concentration was read from the standard curve.

Precision: Within run (intra-assay) variation

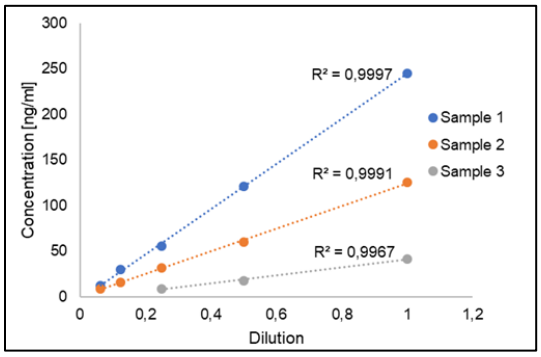
Intra-Assay CV:	5.4 % (224.4 ng/ml)	[n = 10]
	4.5 % (111.9 ng/ml)	[n = 10]
	6.3 % (33.4 ng/ml)	[n = 10]

Precision: Between run (inter-assay) variation

Inter-Assay CV:	6.0 % (227.4 ng/ml)	[n = 10]
	5.0 % (108.4 ng/ml)	[n = 10]
	8.2 % (31.8 ng/ml)	[n = 10]

Linearity

Sample dilution was performed with Wash Buffer.



Sample	Dilution factor	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	-- 1:2	-- 123	245 121	 98.4

	1:4 1:8 1:16	61.3 30.6 15.3	55.6 29.4 12.3	90.8 96.0 80.3
2	-- 1:2 1:4 1:8 1:16	-- 62.3 31.1 15.6 7.8	125 59.4 31.2 15.5 8.5	 95.4 100 99.6 109
3	-- 1:2 1:4	-- 20.6 10.3	41.2 17.7 8.5	 85.9 82.5

Spike and Recovery

Sample	Endogenous [ng/ml]	Added [ng/ml]	Expected [ng/ml]	Measured [ng/ml]	Recovery [%]
1	31.5	22.2 66.6 200	53.7 98.1 232	56.9 106 279	106 108 120
2	112	22.2 66.6 200	135 179 312	128 181 336	94.7 101 108
3	249	22.2 66.6 200	271 316 449	279 353 484	103 112 108

Cross-Reactivity

Cross reactivity to other plasma proteins could not be detected in stool samples.

Limitations of the Method

Stool samples with sIgA concentrations above the standard curve should be diluted with 1x working wash buffer and measured again.

In the case of severe diarrhea, reduced levels can be measured despite an intact state of the gut-associated immune system.

Disposal

The substrate must be disposed of as non-halogenated solvent. The stop solution can be neutralized with NaOH and, if the pH value is neutral, it can be disposed of as salt solution (**important:** this reaction produces heat and should be handled carefully). Please refer to local and national guidelines.

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Referenzbereich: < 0,27 mg/g (2)

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