



**SALIVARY SECRETORY IgA
INDIRECT ENZYME IMMUNOASSAY KIT**

Catalog No. 1-1602, (Single) 96-Well Kit;
1-1602-5, (5-Pack) 480 Wells

For Research Use Only

Intended Use

The Salimetrics™ SIgA kit is an indirect competitive immunoassay designed and validated for the quantitative measurement of SIgA in saliva samples. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Secretory immunoglobulin A (SIgA) is the dominant immunoglobulin in external secretions that bathe mucosal surfaces (respiratory and intestinal) and is often characterized as a component of the immune systems "first-line of defense" against pathogenic microorganisms, viruses, and bacteria (1). SIgA has a parabolic relationship with age. At birth, levels of SIgA are undetectable, but there is a consistent increase in the levels with age. By age 7 years, the levels of SIgA reach their approximate peak. SIgA levels remain consistently high during mid-life and then decline during old age. No gender differences in SIgA levels have been reported. SIgA in saliva is not directly related to serum levels of SIgA (2-5). A lower concentration of SIgA in saliva has been conceptualized as a risk factor for upper respiratory infection in children and the elderly (2-4). Also, individual differences in SIgA levels in response to infection have been identified as a potential risk factor (6). Lower levels of SIgA are associated with increased risk for periodontal disease and caries (7,8). Several studies link stress and emotionality with levels of SIgA (9,10). This literature recommends that variability in salivary flow rate should be taken into account when estimating saliva levels of SIgA and making comparisons between individuals. The present enzyme immunoassay protocol represents a significant advance over the traditional SIgA measurement approach to employing single radial immunodiffusion (SRID). This enzyme immunoassay is designed to capture the full range of salivary SIgA levels and uses only 25 µl of saliva per test, with minimal incubation times.

Test Principle

A constant amount of goat anti-human SIgA conjugated to horseradish peroxidase is added to tubes containing specific dilutions of standards or saliva. The antibody-conjugate binds to the SIgA in the standard or saliva samples. The amount of free antibody remaining is inversely proportional to the amount of SIgA present. After incubation and mixing, an equal solution from each tube is added in duplicate, to microtitre plate coated with human SIgA. The free or unbound antibody conjugate binds to the SIgA on the plate. After incubation, unbound components are washed away. Bound conjugate is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction. Optical density is read on a standard plate reader at 450 nm. The amount of peroxidase is inversely proportional to the amount of SIgA present in the sample (11).

Precautions

1. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.
2. Powdered stop solution is not sulfuric acid-based and is mildly corrosive. Use with care.
3. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
4. Do not mix components from different lots of kits.
5. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
6. See 'Material Safety Data' at the end of procedure.
7. Routine calibration of pipettes is critical for the best possible assay performance.

8. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
9. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
11. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
12. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Microtitre plate:** A ready-to-use, 96-well high affinity binding plate coated with highly purified human SIgA.
2. **SIgA Antibody-Enzyme Conjugate:** 50 µL of a solution of goat anti-human SIgA antibody conjugated to horseradish peroxidase. Dilute prior to use with SIgA diluent.
3. **SIgA Standard:** 100 µL of highly purified human SIgA in a saliva-like matrix with a non-mercury preservative, at a concentration of 600 µg/mL.
4. **SIgA Controls:** Two controls representing high and low levels of SIgA in a saliva-like matrix with a non-mercury preservative. Each vial contains 50 µL.
5. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H₂O). (**Note:** *If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay.*)
6. **SIgA Diluent:** 50 mL of a 5X phosphate buffered solution containing a non-mercury preservative. Dilute 1 part 5X SIgA diluent to 4 parts deionized water (50 mL of 5X SIgA diluent to 200 mL of deionized water). Dilute only the amount needed for current day's use. Discard any leftover reagent.
Note: *Enough SIgA diluent is included to run samples in duplicate only! Please contact Salimetrics to purchase additional SIgA diluent if needed.*
7. **Tetramethylbenzidine (TMB):** 10 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 10 mL of an acidic formulation, lyophilized. Reconstitute with 10 mL of deionized water. Let sit 10 minutes before use.

Materials Needed But Not Supplied

- A precision pipette to deliver 10 µL, 15 µL, 25 µL, 30µL, and 50 µL
- A precision repeater pipette to deliver 50 µL and 100 µL
- A precision multichannel pipette to deliver 50 µL
- Pipette tips
- Small disposable tubes
- 12 x 75 mm snap cap tubes
- One 5 mL serological pipette
- Reagent reservoirs
- Vortex
- Plate rotator with 0.08-0.17" orbit
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water

Specimen Collection

The preferred method for collecting whole saliva is by unstimulated passive drool. Adult samples and samples from children ages 6 and above may also be collected using the Salimetrics Oral Swab (SOS), P/N 5001.02. Collection protocols are available on request. **Do not use Salivettes, cotton, or polyester materials to collect samples.** False readings will result (12). **Do not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected. Avoid sample collection within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Record the time and date of specimen collection. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20°C within 4 hours of collection. (Samples may be stored at -20°C or lower for long term storage.)

Must use IFU that is shipped with product.

Note: Due to the influence that saliva flow rates have on SIgA levels, Salimetrics advises measuring the amount of time needed to collect the desired volume, then using this information to determine the flow rate. The measured concentration of SIgA ($\mu\text{g/mL}$) should then be multiplied by the flow rate (mL/min) to express the results as product measured per unit of time ($\mu\text{g/min}$). If the SOS swab is used to collect the saliva, the length of time the swab was in the mouth should be noted. The amount of saliva collected can be estimated by comparing the weight of the swab and storage tube before and after collecting saliva. The flow rate can then be estimated. In order for the estimate to be valid, the swab must be removed from the mouth before it has absorbed its maximum volume (approximately 2 mL).

Although the Sorbette collection device does not cause interference with the SIgA assay, we advise that it is not a method of choice for SIgA studies because of problems in correcting the assay results for flow rate. Due to the very small volume collected by this device (200-300 μL), we feel that it is probably not possible to reliably estimate the time that was required to reach saturation. Attempts to calculate the flow rate and to correct the assay results for flow rate are therefore likely to be inaccurate.

Concentrations of SIgA also vary significantly depending on the location in the mouth; consistency in collection location is therefore important(13). We find that placing the SOS underneath the tongue on the floor of the mouth yields results similar to those from whole saliva collected by passive drool. Under certain conditions, however, there is a possibility that the SOS might collect specific glandular saliva. Researchers should be aware of this potential and decide on their collection strategy accordingly.

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. It is important to avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. **Note:** *It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.*

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	Ctrl 1	Ctrl 1								
B	S2	S2	Ctrl 2	Ctrl 2								
C	S3	S3	Unk-1	Unk-1								
D	S4	S4	Unk-2	Unk-2								
E	S5	S5	Unk-3	Unk-3								
F	S6	S6	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB*	NSB*	Unk-6	Unk-6								

*NSB = Non-specific binding wells. These may serve as blanks. Use is optional.

Step 2: Keep the desired number of wells in the strip holder and place the remaining strips back in the pouch. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Step 3:

- Label five microcentrifuge tubes or other small tubes 2 through 6.
- Pipette 30 μL of 1X SIgA diluent in tubes 2 through 6. Serially dilute the standard 3X by adding 15 μL of the 600 $\mu\text{g/mL}$ standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 15 μL from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6, are respectively, 600 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$, 66.7 $\mu\text{g/mL}$, 22.2 $\mu\text{g/mL}$, 7.4 $\mu\text{g/mL}$, and 2.5 $\mu\text{g/mL}$.
- Pipette 3 mL of 1X SIgA diluent into a tube. (Scale down proportionally if not using entire plate.) Set aside for Step 6.

Step 4:

- Label one small tube with the identity of each saliva sample. (Do not pre-dilute controls 5X.)
- With a repeater pipette, add 100 μL of 1X SIgA diluent into each tube.
- Pipette 25 μL of saliva into the appropriate tube. (If saliva sample is less than 25 μL , use 10 μL of saliva to 40 μL of diluent.)
- Note:** *If results are below the low limit of sensitivity, samples should be repeated straight (eliminating the 5X dilution). In this case, do not multiply the final results x 5.*

Step 5:

- Label one 12 x 75 mm snap-cap tube for each standard, control, and unknown sample, and one tube for the zero value.
- Using a repeater pipette, add 4 mL of 1X SIgA diluent to each tube.
- Add 10 μL of standard (from step 3), control, or diluted unknown saliva sample (from step 4) to the appropriate tube.
- Add 10 μL of 1X SIgA diluent to the zero tube.

Step 6:

- Dilute the antibody-enzyme conjugate 1:120 by adding 25 μL of the conjugate to the 3 mL of 1X SIgA diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Conjugate in the microcentrifuge tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom.
- Mix well and pipette 50 μL of the diluted antibody-enzyme conjugate to all tubes using a repeater pipette.
- Gently mix each tube by inversion and incubate for 90 minutes at room temperature.

Step 7:

- Gently mix each tube by inversion again and add 50 μL of solution from step 6 to the microtitre plate according to your template.
- If using NSB wells, add 50 μL of 1X SIgA diluent to those 2 wells.
- Cover plate with the adhesive plate sealer and incubate at room temperature with continual mixing at 400 rpm for 90 minutes.

Step 8: Wash the plate 6 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently adding wash buffer into each well with a squirt bottle, or by pipetting 300 μL of wash buffer into each well and then decanting the liquid into a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blot thoroughly after the last wash.

Step 9: Add 50 μL of TMB solution to each well with a multichannel pipette

Step 10: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark at room temperature for an additional 40 minutes.

Step 11:

- Add 50 μL of stop solution with a multichannel pipette.
- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

- Compute the average optical density (OD) for all duplicate wells.
- Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
- Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
- Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.
- Multiply concentrations of unknown saliva samples by 5 to obtain the final concentration of SIgA in $\mu\text{g/mL}$.**

Quality Control

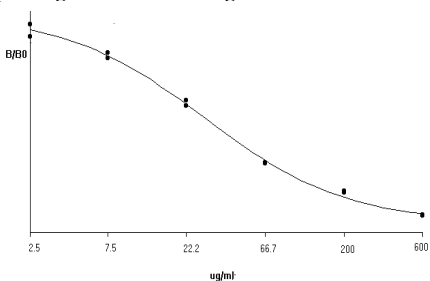
The Salimetrics' high and low salivary SIgA controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	B	B/Bo	SIgA ($\mu\text{g/mL}$)
A1,A2	S1	0.140	0.130	0.079	600.0
B1,B2	S2	0.313	0.303	0.185	200.0
C1,C2	S3	0.526	0.516	0.314	66.7
D1,D2	S4	0.970	0.960	0.585	22.2
E1,E2	S5	1.326	1.316	0.801	7.4
F1,F2	S6	1.509	1.499	0.913	2.5
G1,G2	Bo	1.652	1.642	NA	0
H1,H2	NSB	0.010	NA	NA	NA

Example: SIgA 4-Parameter Sigmoid minus Curve Fit



Material Safety Data*

Hazardous Ingredients

Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive. Use with care.

We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics shall not be liable for accidents or damage resulting from contact with reagents.

Performance Characteristics

A. Recovery:

Six saliva samples containing different levels of endogenous SIgA were spiked with known quantities of SIgA and assayed.

Sample	Endogenous (µg/mL)	Added (µg/mL)	Expected (µg/mL)	Observed (µg/mL)	Recovery (%)
1	88.77	250.00	338.77	377.8	111.5
2	79.42	90.00	169.42	176.96	104.5
3	84.1	20.00	104.1	120.7	115.9
4	275.96	250.00	525.96	529.7	100.7
5	285.6	90.00	375.6	394.68	105.1
6	302.4	20.00	322.4	358.73	111.3

B. Precision:

The intra-assay precision was determined from the mean of 10 replicates each.

Sample	N	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
H	10	805.38	56.32	6.99
M	10	336.03	17.89	5.32
L	10	91.08	4.09	4.49

The inter-assay precision was determined from the mean of average duplicates for 8 separate runs.

Sample	N	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
H	8	204.10	17.65	8.65
L	8	25.33	2.26	8.93

C. Method Comparison

Inter-method correlations for SIgA levels from saliva samples (n = 21) assayed using the present EIA protocol and a radial immunodiffusion assay, and the present protocol and a commercially available SIgA ELISA, were $r(19) = 0.94$ and 0.91 , $p < 0.0001$, respectively. The SIgA levels returned by the present EIA protocol ($M = 379.39$ µg/mL; $St Dev = 261.47$) and the comparison ELISA ($M = 365.81$ µg/mL; $St Dev = 311.53$) were not statistically distinct. SIgA levels returned by radial immunodiffusion were significantly higher ($M = 675.21$; $St Dev = 467.94$) than both immunoassay protocols.

D. Linearity Of Dilution:

Three saliva samples were diluted with SIgA diluent and assayed.

Sample	Dilution Factor	Expected (µg/mL)	Observed (µg/mL)	Recovery (%)
Sample 1			364.60	
	1:2	182.3	198.40	108.8
	1:4	91.15	87.76	96.3
	1:8	45.58	39.80	87.3
Sample 2	1:16	22.79	19.92	87.4
			456.70	
	1:2	228.35	262.35	114.9
	1:4	114.18	129.45	113.4
Sample 3	1:8	57.09	51.45	90.1
	1:16	28.54	32.00	112.1
			389.36	
	1:2	194.68	207.04	106.3
	1:4	97.34	95.04	97.6
	1:8	48.67	50.32	103.4
	1:16	24.34	23.84	97.9

E. Sensitivity:

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 18 replicates at the 0 µg/mL level. The minimal concentration of SIgA that can be distinguished from 0 is 2.5 µg/mL.

Salivary SIgA Expected Ranges

Group	Number	Mean (µg/mL)	Std Dev (µg/mL)
Adults	21	379.39	261.47

References

1. Tomasi, T.B. Jr. (1976). *The immune system of secretions*. Englewood Cliffs, NJ: Prentice-Hall.
2. Ben-Aryeh, H., Fisher, M., Szargel, R., & Laufer, D. (1990). Composition of whole unstimulated saliva of healthy children: Changes with age. *Archives of Oral Biology*, 35, 929-931.
3. Smith, D.J., Taubman, M.A., & Ebersole, J.L. (1987). Ontogeny and senescence of salivary immunity. *Journal of Dental Research*, 66, 451-456.
4. Ventura, M.T. (1991) Evaluation of IgA-1 - IgA-2 levels in serum and saliva of young and elderly people. *Allergol Immunopathol (madr)*, 19, 183-185.
5. Kugler, J., Hess, M., & Haake, D. (1992). Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. *Journal of Clinical Immunology*, 12, 45-49.
6. Jemmott III, J.B. & McClelland, D.C. (1989). Secretory IgA as a measure of resistance to infectious disease: Comments on Stone, Cox, Valdimarsdottir, and Neale. *Behavioral Medicine*, 15, 63-71.
7. Gregory, R.I., Kim, D.E., Kindle, J.C., Hobbs, L.C., & Lloyd, D.R. (1992). Immunoglobulin-degrading enzymes in localized juvenile periodontitis. *Journal of Periodontal Research*, 27, 176-183.
8. Ruan, M.S. (1990). The secretory IgA and caries. *Chung-Hua-Kou-Chiang-Hsueh-Tsa-Chin*, 25, 158-160.
9. Jemmott III, J.B., & Magloire, K. (1988). Academic stress, social support, and secretory immunoglobulin A. *Journal of Personality and Social Psychology*, 55, 803-810.
10. Kugler, J. (1991). Mood and salivary immunoglobulin A: A review. *Psychotherapy, Psychosomatic Medicine and Psychology*, 41, 232-242.
11. Chard, T. (1990). *An introduction to radioimmunoassay and related techniques* (4th ed.). Amsterdam: Elsevier.
12. Shirtcliff, E.A., Granger, D.A., Schwartz, E., & Curran, M.J. (2001). Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology*, 26, 165-173.
13. Brandtzaeg, P. (2007). Do salivary antibodies reliably reflect both mucosal and systemic immunity? *Ann NY Acad Sci*, 1098, 288-311.

Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."