YK280 Human s-IgA (Saliva) ELISA

FOR LABORATORY USE ONLY

YANAIHARA INSTITUTE INC.
2480-1 AWAKURA, FUJINOMIYA-SHI
SHIZUOKA, JAPAN 418-0011

Contents

– Please read all the package insert carefully before beginning the assay –

YK280 Human s-IgA (Saliva) ELISA

Ⅰ. Introduction

Secretory IgA (s-IgA) is the main effector of the mucosal immune system and act as the first line of immune mechanisms at the mucosal surface. Secretory IgA is included in the most abundant immunoglobulin of body sccretions such as saliva, tears, colostrum and gastrointestinal secretions. Secretory IgA is produced by IgA-producing plasma cells predominantly as polymeric IgA consisting of dimer linked by J chain. The polymeric IgA is transported by the epithelial polymeric immunoglobulin receptor and released into mucosal secretions with a bound secretory component (SC).
Since the collection of saliva is capable of easy and non-invasive sampling, s-IgA concentrations in saliva has been used as a useful biomarker in various stresses, psychological studies [1 and 2, 3)].
The kit can be used for measurement of human s-IgA in saliva with high sensitivity. It will be a specifically useful tool for various stresses and psychological studies.

YK280 Human s-IgA (Saliva) ELISA Kit

- ▼ The assay kit can measure s-IgA in saliva within the range of 0.082～20 μg/mL
- ▼ The assay is completed within 1hr+1hr+0.5hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: Human saliva
  Sample volume: 5 μL
- ▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.
- ▼ Stability and storage
  Store all of the components at 2-8ºC.
  The kit is stable under the condition for 24 months from the date of manufacturing.
  The expiry date is stated on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) HRP labeled antibody solution
4) Enzyme substrate solution (TMB)
5) Stopping solution
6) Buffer solution (concentrated 2x)
7) Washing solution (concentrated)
8) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of human secretory IgA (s-IgA) in saliva. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. Human s-IgA standard is highly purified product.

< Specificity >
This ELISA kit has high specificity to human s-IgA, and shows no cross reactivity to human serum IgA, serum IgG, serum IgM and serum IgE.

< Assay principle >
This ELISA kit for determination of human s-IgA is based on a sandwich enzyme immunoassay. To the wells of plate coated with highly purified mouse monoclonal antibody against human s-IgA, standards or samples are added for the 1st step immunoreaction. After the 1st step incubation and plate washing, HRP labeled antibody solution against human IgA (α chain) is added as the 2nd step to form antibody - antigen - labeled antibody complex on the surface of the wells. After the 2nd step incubation and rinsing out excess labeled antibody, Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of human s-IgA is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti human s-IgA monoclonal antibody coated
2.	Standard	lyophilized	1 vial (10 μg)	Purified human s-IgA
3.	HRP labeled antibody solution	liquid	1 bottle (12 mL)	HRP labeled goat anti human IgA (α chain) antibody
4.	Enzyme substrate solution (TMB)	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
5.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
6.	Buffer solution (concentrated 2x)	Liquid	1 bottle (25 mL)	Buffer containing a reaction accelerator
7.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
8.	Adhesive foil		3 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader) which can read extinction 3.0 at 450nm
2. Microtiter plate shaker
3. Washing device for microtiter plate and dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Glass test tubes for preparation of standard solution and saliva sample dilution
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

<Preparation of saliva sample>

Saliva samples may be collected using the SalivaBio Oral Swab (Item No. 5001.02 Salimetrics) and Swab storage tube (Item No. 5001.05 Salimetrics). Saliva collection should be done according to the instruction manuals. After saliva collection, the tube is centrifuged at 3000 rpm for 15 min. And the sample should be divided into test tubes in small amount and frozen at or below -30℃ until assay. These samples should be brought back to room temperature (20-30°C) before starting assay. If insoluble material is observed in sample, they should be removed by centrifugation（3000 rpm x 15 min.） and the sample solution is submitted to assay immediately.

< Preparatory work >

1. Preparation of 1x buffer solution
   Dilute buffer solution (concentrated, 2x) (25 mL) to 50 mL with distilled water. Dilute only the amount needed for current assay.
2. Preparation of standard solution:
   Reconstitute the human s-IgA standard with 0.5 mL of buffer solution and stand for about 10 min., which affords 20 μg/mL standard solution. The reconstituted standard solution (0.1 mL) is diluted with 0.2 mL of buffer solution that yields 6.67 μg/mL standard solution. Repeat the dilution procedure to make each standard solution of 2.22, 0.74, 0.25 and 0.082 μg/mL. Buffer solution itself is used as 0 μg/mL standard solution.
3. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled water.

4. Other reagents are ready for use.

<Dilution of saliva sample (1 : 80 dilution)>

Add 395 μL of buffer solution into an appropriate tube. Pipette 5 μL of saliva into the tube and vortex to mix.

< Procedure >

1. Before starting the assay, bring all the reagents and samples to room temperature (20 ~ 30ºC).

2. Fill 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

3. Add 100μL of buffer solution to the wells first, and then introduce 50μL of each of standard solutions (0, 0.082, 0.25, 0.74, 2.22, 6.67 and 20 μg/mL) or samples to the wells.

4. Mix the plate with a plate mixer for 1 min at approximately 500 rpm. without adhesive foil. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

5. After incubation, take off the adhesive foil, aspirate and wash the wells 6 times with 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

6. Add 100 μL of HRP labeled antibody solution to each of the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

8. Take off the adhesive foil, aspirate and wash the wells 6 times with 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

9. Add 100μL of Enzyme substrate solution (TMB) to each of the well, cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction (keep still, plate shaker not need).

10. Add 100 μL of stopping solution to each of the wells to stop color reaction.

11. Read the optical absorbance of the solution in the wells at 450 nm/620 nm. The dose-response curve

of this assay fits best to a 5 (or 4)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5 (or 4)-parameter logistic function. Otherwise calculate mean absorbance values of wells containing standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Multiply concentrations of unknown samples by 80 to obtain the final concentration of s-IgA.

Ⅴ. Notes

1. Saliva samples should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples.

2. Standard solutions should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagent (standard) should be stored at or below -30°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however, they will be dissolved when diluted.

4. Pipetting operations may affect the precision of the assay, so that pipette standard solutions or samples precisely into each well of plate. In addition, use clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.

5. When sample concentration exceeds 20 μg/mL, it needs to be diluted with buffer solution to proper concentration.

6. During the incubation except the color reaction, the plate should be shaken gently by a plate shaker to promote immunoreaction (approximately 100 rpm).

7. Perform all the determination in duplicate.

8. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.

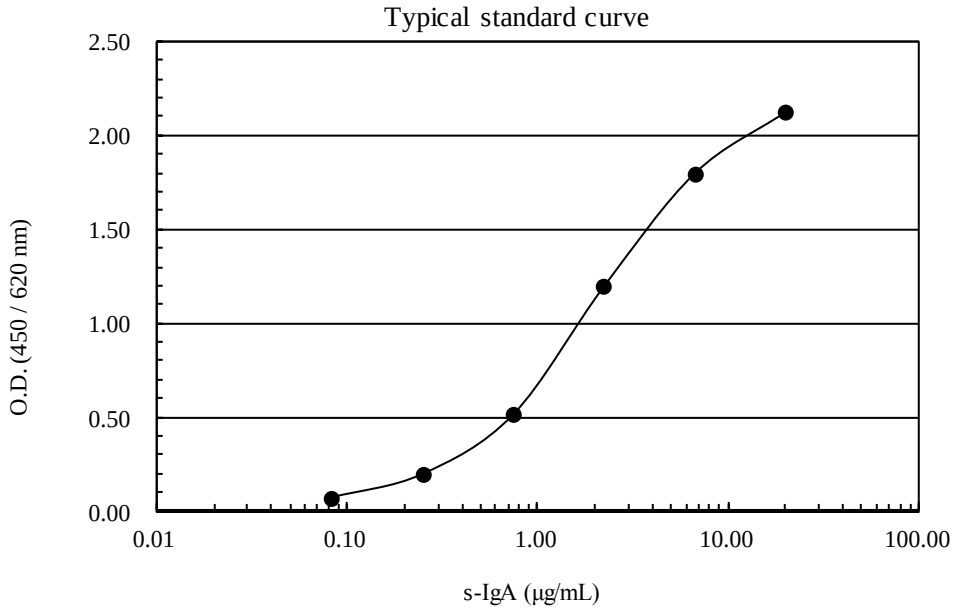
9. To quantitate accurately, always run a standard curve when testing samples.

10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

11. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

12. Human s-IgA standard is purified from human colostrum, care should be taken when handling.

Ⅵ. Performance Characteristics



<Analytical Recovery>

<Human saliva A>

Added s-IgA (μg/mL)	Observed (μg/mL)	Expected (μg/mL)	Recovery (%)
0	64.8		
40	108.8	104.8	103.8
160	220.8	224.8	98.2
400	404.8	464.8	87.1

< Human saliva B>

Added s-IgA (μg/mL)	Observed (μg/mL)	Expected (μg/mL)	Recovery (%)
0	46.4		
40	96.0	86.4	111.1
160	196.0	206.4	95.0
400	432.0	446.4	96.8

< Human saliva C>

Added s-IgA (μg/mL)	Observed (μg/mL)	Expected (μg/mL)	Recovery (%)
0	120.8		
40	189.6	160.8	117.9
160	274.4	280.8	97.7
400	476.0	520.8	91.4

< Human saliva D>

Added s-IgA (μg/mL)	Observed (μg/mL)	Expected (μg/mL)	Recovery (%)
0	112.0		
40	157.6	152.0	103.7
160	244.8	272.0	90.0
400	441.6	512.0	86.3

< Human saliva E>

Added s-IgA (μg/mL)	Observed (μg/mL)	Expected (μg/mL)	Recovery (%)
0	105.6		
40	152.0	145.6	104.4
160	250.4	265.6	94.3
400	600.8	505.6	118.8

<Dilution test >

< Human saliva A>

Sample dilution	Observed (μg/mL)	Expected (μg/mL)	% of Expected (%)
X1	363.5	363.5	
X2	201.7	181.8	111.0
X4	107.7	90.9	118.5
X8	53.6	45.4	117.9

< Human saliva B>

Sample dilution	Observed (μg/mL)	Expected (μg/mL)	% of Expected (%)
X1	237.4	237.4	
X2	123.9	118.7	104.4
X4	65.3	59.4	110.0
X8	32.5	29.7	109.4

< Human saliva C>

Sample dilution	Observed (μg/mL)	Expected (μg/mL)	% of Expected (%)
X1	310.0	310.0	
X2	167.3	155.0	107.9
X4	89.5	77.5	115.5
X8	42.7	38.8	110.2

< Human saliva D>

Sample dilution	Observed (μg/mL)	Expected (μg/mL)	% of Expected (%)
X1	293.3	293.3	
X2	145.3	146.7	99.1
X4	74.7	73.3	101.8
X8	38.3	36.7	104.4

< Human saliva E>

Sample dilution	Observed (μg/mL)	Expected (μg/mL)	% of Expected (%)
X1	188.2	188.2	
X2	97.5	94.1	103.6
X4	47.4	47.0	100.7
X8	26.8	23.5	114.0

<Crossreactivity>

Related antibodies	Crossreactivity（%）
Serum IgG (Human)	<0.1
Serum IgA (Human)	0.3
Serum IgM (Human)	0.0
Serum IgE (Human)	0.0

< Precision and reproducibility >

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Human saliva	2.9~ 5.8	3.5~ 11.2

<Assay range>

0.082 ~ 20 μg/mL

<Correlation with other EIA kit for saliva >

The correlation with the measured values obtained using other EIA kit for saliva (Salimetrics) is as follows.

y=0.8539x + 31.222, r=0.946 (n=39)

Ⅶ. Stability and Storage

< Storage > Store all of the components at 2-8°C.

< Shelf life > The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is stated on the label of kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

1. Evans, P., Bristow, M., Hucklebridge, F., Clow, A., and Pang, FY. (1994) Stress, arousal, cortisol and secretory immunoglobulin A in students undergoing assessment. Br. J. Clin. Psychol., 33, 575-576
2. Bristow, M. Hucklebridge, F., Clow, A., and Evans, P. (1997) Modulation of secretory immunoglobulin A in saliva in relation to an acute episode of stress and arousa. J. Psychophysiol., 11, 248-255
3. Fujiwara, S., Yogo, M. (2003) Measuring salivary immunoglobulin A for psychological studies of stress and emotion. Doshisha Psychological Review, 50, 57-81

<Manufacturer>
Yanaihara Institute Inc.
2480-1 Awakura, Fujinomiya-shi
Shizuoka, Japan 418-0011
TEL: +81-544-22-2771　FAX: +81-544-22-2770
Website: http://www.yanaihara.co.jp　E-mail: ask@yanaihara.co.jp

Update at July 19, 2017