YK340 Substance P (Saliva) EIA

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 -

YK340 Substance P (Saliva) EIA Kit

Ⅰ. Introduction

Substance P is a peptide composed of a chain of 11 amino acid residues, which is one of a member of neuropeptides with similar structure called tachykinin.
Substance P was first found to function as a neurotransmitter, and subsequent research has shown that it is expressed not only in the central and peripheral nervous systems, but also in peripheral non-nervous cells, and is now involved in a wide range of physiological functions. It is also known to be associated with various pathologies.
Pneumonia is considered to be one of the major causes of death among the elderly in Japan, and decreased secretion of substance P leads to a decrease in normal swallowing reflex and cough reflex, which increases silent aspiration, it has been pointed out that the risk of aspiration pneumonia is increased as a result.
Saliva can be collected easily and noninvasively, and Substance P concentration in saliva is considered to be a useful biomarker.
This kit can specifically and easily measure Substance P present in saliva.

YK340 Substance P (Saliva) EIA Kit

- ▼ The assay kit can measure Substance P in saliva within the range of 6.859-5,000 pg/mL
- ▼ The assay is completed within 18 hr.+1.5 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate.
- ▼ Test sample: Human saliva
  Sample volume: 50 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 18 months from the date of manufacturing.
  The expiry date is stated on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antigen
4) Antibody solution
5) SA-HRP solution
6) Enzyme substrate solution (TMB)
7) Stopping solution
8) Buffer solution
9) Washing solution (concentrated)
10) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of Substance P in saliva. The kit is characterized by sensitive quantification and high specificity. In addition, it has no influence by other constituents in samples. Substance P standard of this kit is a highly purified synthetic product.

< Assay principle >

This EIA kit for determination of Substance P is based on a competitive enzyme immunoassay using combination of highly specific antibody to Substance P and biotin-avidin affinity system. To the well of the plate coated with goat anti rabbit IgG, Substance P standard or samples, labeled antigen and Substance P antibody are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptavidin (SA) is added to form HRP labeled SA-biotinylated Substance P - antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of Substance P is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG antibody
2.	Standard	lyophilized	1 vial (5,000 pg)	Substance P (1-11) amide
3.	Labeled antigen	lyophilized	1 vial	Biotinylated Substance P
4.	Antibody solution	liquid	1 bottle (7 mL)	Rabbit anti Substance P (1-11) amide antibody
5.	SA-HRP solution	liquid	1 bottle (12 mL)	Horseradish peroxidase labeled streptavidin
6.	Enzyme substrate solution (TMB)	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
7.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
8.	Buffer solution	liquid	1 bottle (30 mL)	Phosphate buffer
9.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10.	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>

To collect saliva, use an oral swab + storage tube (Funakoshi Co., Ltd. product code 5001.02 + 5001.05). Collect saliva according to the instruction manual of the collection device. Collected saliva samples should be frozen at or below -30°C immediately. (If possible, cryopreservation of saliva samples is recommended at -80°C.) These samples should be brought back to room temperature (20-30°C), and then be centrifuged (3000 rpm x 15 min.) before starting assay.

< Preparatory work >

1. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 7 mL of buffer solution.
2. Preparation of standard solution:
   Reconstitute the standard with 1.0 mL of buffer solution, which affords 5000 pg/mL standard solution. The reconstituted standard solution (0.1 ml) is diluted with 0.2 mL of buffer solution, which yields 1666.667 pg/mL standard solution. Repeat the dilution procedure to make each of 555.556, 185.185, 61.728, 20.576 and 6.859 pg/mL standard solutions. Buffer solution itself is used as 0 pg/mL.
3. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000mL with distilled water.
4. Other reagents are ready for use.

<Dilution of saliva sample>

Thaw frozen saliva samples at room temperature. Centrifuge (3,000 rpm, 15 minutes) and well mix collected samples. Dilute these saliva samples with buffer 4 times.
< Example of dilution >
Add 150 μL of buffer solution into an appropriate tube. Pipette 50 μL of saliva into the tube and vortex to mix.

< Procedure >

1. Bring all the reagents and samples to room temperature (20-30ºC) at least 1 hour before starting assay.

2. Add 0.35 mL/well of washing solution into the wells and aspirate 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. Fill 50 μL of each of standard solutions (0, 6.859, 20.576, 61.728, 185.185, 555.556, 1666.667, 5000 pg/mL) or samples into the wells first, then introduce 50 μL of labeled antigen solution and finally add 50 μL of antibody solution into the wells.

4. Cover the plate with adhesive foil and incubate it at room temperature for 18 hours. 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 5 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 SA-HRP solution into 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 5 times with approximately 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 100 μL 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 into 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 4 times to obtain the final concentration of Substance P.

V. Notes

1. The measurement is affected by surfactants, so be careful not to contaminate such as toothpaste or mouthwash into saliva sample. It has been confirmed that there is a tendency for high absorbance at the measurement, due to the interference effect of the surfactants.

2. 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. If possible, cryopreservation of saliva samples is recommended at -80°C.

3. Precipitation may occur in buffer solution and antibody solution, please tipping agitation before use. The precipitate has no effect on the measurement results.

4. 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 and labeled antigen) should be stored at 4°C. The reconstituted standard (5,000 pg/mL) and the labeled antigen are stable for 4 weeks when stored refrigerated at 4°C. If stored these reagents frozen at -30°C, the performance is stable for 4 weeks, but many precipitate are generated with freezing, it is not recommended to store reagents frozen.

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

6. 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.

7. When sample concentration exceeds 5,000 pg/mL, it needs to be diluted with buffer solution to proper concentration.

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

9. Perform all the determination in duplicate.

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

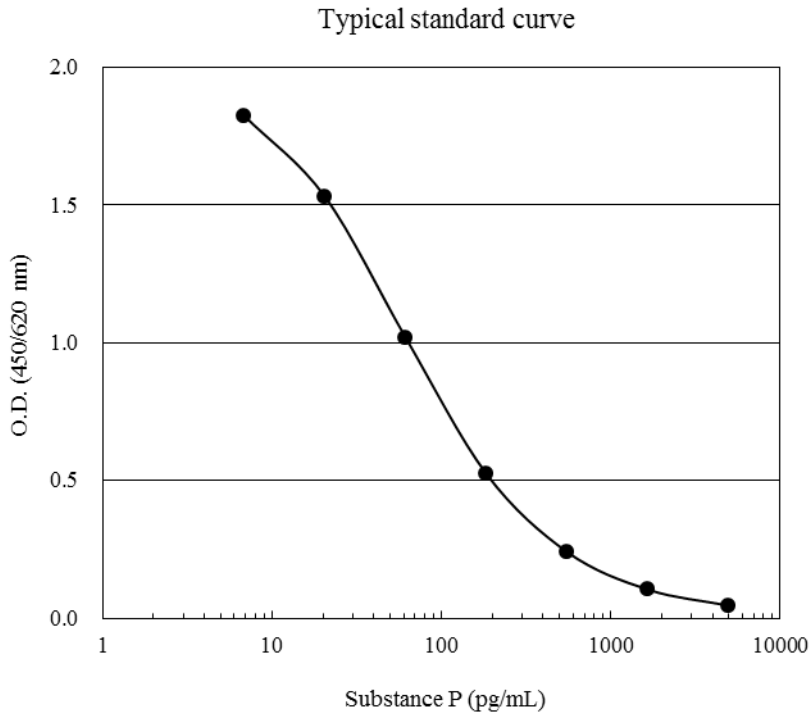
reaction.

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

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

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

Ⅵ. Performance Characteristics



<Analytical Recovery>

<Human saliva A>

Added Substance P (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	21.6		
15	36.1	36.6	98.6
200	240.9	221.6	108.7
1,000	1004.1	1021.6	98.3

<Human saliva B>

Added Substance P (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	42.1		
15	64.3	57.1	112.6
200	273.4	242.1	112.9
1,000	1033.6	1042.1	99.2

<Human saliva C>

Added Substance P (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	25.2		
15	42.6	40.2	106.0
200	272.2	225.2	120.9
1,000	1020.9	1025.2	99.6

<Human saliva D>

Added Substance P (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	11.7		
15	27.4	26.7	102.6
200	232.2	211.7	109.7
1,000	1068.0	1011.7	105.6

<Human saliva E>

Added Substance P (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	20.6		
15	41.9	35.6	117.7
200	277.4	220.6	125.8
1,000	1249.8	1020.6	122.5

<Dilution test >

Let x1 be a saliva sample before dilute preparation. x3 is a reference value.

< Human saliva A>

Sample preparation	Observed (pg/mL)	Expected (pg/mL)	% of Expected (%)
x3	36.1	108.3	123.1
x4	22.0	88.0	
x6	13.7	82.2	93.4
x8	10.0	80.0	90.9

< Human saliva B>

Sample preparation	Observed (pg/mL)	Expected (pg/mL)	% of Expected (%)
x3	61.4	184.2	111.0
x4	41.5	166.0	
x6	27.3	163.8	98.7
x8	21.0	168.0	101.2

< Human saliva C>

Sample preparation	Observed (pg/mL)	Expected (pg/mL)	% of Expected (%)
x3	43.9	131.7	119.7
x4	27.5	110.0	
x6	17.4	104.4	94.9
x8	13.2	105.6	96.0

< Human saliva D>

Sample preparation	Observed (pg/mL)	Expected (pg/mL)	% of Expected (%)
x3	22.4	67.2	108.4
x4	15.5	62.0	
x6	9.6	57.6	92.9
x8	6.9	55.2	89.0

<Human saliva E>

Sample preparation	Observed (pg/mL)	Expected (pg/mL)	% of Expected (%)
x3	32.7	98.1	110.5
x4	22.2	88.8	
x6	13.6	81.6	91.9
x8	11.7	93.6	105.4

< Precision and reproducibility >

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Human saliva	2.8-5.6	6.1-10.9

<Crossreactivity>

Related peptides	Crossreactivity（%）
Substance P (1-4)	< 0.1
Substance P (3-11) amide	86.5
Substance P (4-11) amide	99.1
Substance P (7-11) amide	48.8
Neurokinin A	0.3
Neurokinin B	0.1
Hemokinin-1 (human)	86.2

Ⅶ. Stability and Storage

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

< Shelf life > The kit is stable under the condition for 18 months from the date of manufacturing. The expiry date is stated on the package.

< Package > For 96 tests per one kit.

Ⅷ. References

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<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 Dec. 27, 2022