

IgA Saliva ELISA

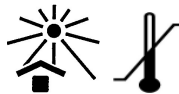
Enzyme immunoassay for the *in-vitro diagnostic* determination of human IgA in saliva.

REF

DM59171



96



2-8 °C

EU: **IVD**



U.S.: *For research use only.
Not for use in diagnostic procedures.*



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1. INTENDED USE

Enzyme immunoassay for the in-vitro diagnostic determination of human IgA in saliva.

2. CLINICAL SIGNIFICANCE

IgA represents about 15% to 20% of immunoglobulins in the blood, they are also found in the mucus secreted in the stomach, lungs and intestines. This prevents the microbes to bind to epithelial cells of the respiratory and digestive tract. This immunoglobulin helps to fight against pathogens that contact the body surface, are ingested, or are inhaled. It exists in two forms, IgA1 (90%) and IgA2 (10%) that differ in the structure. IgA1 is found in serum and made by bone marrow B cells, however IgA2 is made by B cells located in the mucosae and has been found to secrete into, colostrum, maternal milk, tears and saliva.

The IgA found in secretions have a special form. They are dimeric molecules, linked by two additional chains. One of these is the J chain (from *join*), which is a polypeptide of molecular mass 1.5 kDa, rich with cysteine and structurally completely different from other immunoglobulin chains. The dimeric form of IgA in the outer secretions also has a polypeptide of the same molecular mass (1.5 kDa) called the secretory chain and is produced by epithelial cells. Decreased or absent IgA, termed *selective IgA deficiency*, can be a clinically significant immunodeficiency.

3. PRINCIPLE

The h-IgA saliva ELISA TEST is based on simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on microwell plates, the other, polyclonal conjugates with horseradish peroxidase (HRP).

After incubation the bound/free separation is performed by a simple solid-phase washing.

The enzyme in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.

The color intensity is proportional to the IgA concentration in the sample.

The IgA concentration in the sample is calculated based on a standard curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Coated Microplate **MTP**
(1 breakable microplate) Antibody anti IgA adsorbed on microplate
2. 20X Conc. Conjugate **ENZCONJ CONC**
(1 vial, 1 mL) Antibody anti IgA conjugated with horseradish peroxidase (HRP)
3. IgA Standards **CAL 0-4**
(5 vials, 1 mL each)
4. IgA saliva Control **CONTROL**
(1 vial, 1 mL) Concentration of Control is Lot-specific and is indicated on Quality Control Report
5. 5X Conc. IgA Assay Buffer **ASSAYBUF CONC**
(1 vial, 40 mL) Hepes buffer 25 mM pH 7.4; BSA 0,5 g/L
6. TMB Substrate **TMB SUBS**
(1 vial, 15 mL) H_2O_2 -TMB 0.26 g/L (avoid any skin contact)
7. 50X Conc. Wash Solution **WASHBUF CONC**
(1 vial, 20 mL) NaCl 45 g/L; Tween-20 55 g/L
8. Stop Solution **STOP**
(1 vial, 15 mL) Sulphuric acid 0.15 mol/L (avoid any skin contact)

4.2. Reagents necessary not supplied

Distilled water.

4.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Note

Store all reagents between 2 – 8 °C in the dark.

Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use.

Do not remove the adhesive sheets on the strips unutilized.

5. WARNINGS

- This kit is intended for in vitro use by professional persons only.
- Use appropriate personal protective equipment while working with the reagents provided.
- Some reagents contain small amounts of Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants.
- This method allows the determination of IgA from 0.5 µg/mL to 400 µg/mL.

6. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- All reagents should be stored refrigerated at 2 - 8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22 - 28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated should not be used in the assay. Highly lipemic or haemolysed specimens should similarly not be used.
- Plate readers measure vertically. Do not touch the bottom of the wells.

7. PROCEDURE

7.1. Preparation of the Standards (S0...S4)

Standards and Control are ready for use.

The Standards have the following concentration: 0; 6.9; 62; 132; 400 ng/mL.

Once opened, the standards are stable six months at 2 - 8°C.

The standard concentration are 1000 times lower than the values reported in the reference range because the samples are diluted 1:1000 while the standards are not diluted.

The concentrations of Standards to be entered in the instrument for calculations are:

	S ₀	S ₁	S ₂	S ₃	S ₄
µg/mL	0	6.9	62	132	400

7.2. Preparation of IgA Assay Buffer

Dilute contents of 5X Conc. IgA Assay Buffer with 160 mL of distilled or deionized water in a suitable storage container. To prepare different volumes respect the dilution ratio 1:5. Store at 2 - 8°C until the expiry date printed on the label.

7.3. Preparation of Diluted Conjugate

Prepare immediately before use.

Add 50 µL of conjugate (reagent 4) to 950 µL of diluted IgA Assay Buffer (reagent 3). The quantity of diluted conjugate is proportional at the number of tests. Mix gently for 5 minutes, with rotating mixer. Stable for 3 hours at room temperature (22 - 28°C).

7.4. Preparation of Wash solution

Dilute the content of 50X Conc. Wash Solution to 1000 mL with distilled or deionized water in a suitable storage container. Store at room temperature (22 - 28°C) until expiry date printed on the label.

7.5. Preparation of the Sample

For sample collection is advised to use a centrifuge glass tube and a plastic straw.

Let the saliva flow down through the straw into the centrifuge glass tube; then centrifuge at 3000 rpm per 15 minutes. Do not use plastic tube or commercially available devices for the saliva collection to avoid false results.

Prepare the A solution for each sample by diluting supernatant liquid 1:20 with diluted Assay Buffer (ie: 50 µL up to 1 mL); then mix gently every A solution by leaving it for at least 5 minutes on a rotating shaker and dilute this 1:50 with diluted Assay Buffer (ie: 20 µL up to 1 mL). Finally dilution obtained: 1:1000.

Mix gently by leaving it for at least 5 minutes on a rotating shaker.

If the assay is not carried out in the same day of collection store the saliva at -20°C.

7.6. Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0 - S_4), two for each sample, two for Control, one for Blank.

Reagent	Standard	Sample/ Control	Blank
Standard S0-S4	25 µL		
Diluted Samples/Control		25 µL	
Diluted Conjugate	100 µL	100 µL	
Incubate 1 hour at room temperature (22 - 28°C). Remove the contents from each well; wash the wells three times with 300 µL of diluted wash solution.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22 - 28°C).			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank			

8. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of IgA for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

9. RESULTS

9.1. Mean Absorbance

Calculate the mean of the absorbance (E_m) for each point of the standard curve and of each sample.

9.2. Calculation of Results – Automatic method

To use the method: 4 parameter logistic, sigmoid logistic or smoothed cubic spline like calculation algorithm.

9.3. Calculation of Results – Manual method

A dose response curve is used to ascertain the concentration of IgA in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader.
- Plot the absorbance for each duplicate serum reference versus the corresponding IgA concentration in µg/mL on linear graph paper.
- Connect the point with a best-fit curve.
- To determine the concentration of IgA for unknown samples, locate the average absorbance of the duplicates for each unknown sample on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

10. REFERENCE VALUES

Based on the literature data and on the results obtained with the h-IgA IBL kit, an highly summarized range of normality is: 40 - 170 µg/mL

11. PERFORMANCE AND CHARACTERISTICS

11.1. Sensitivity

The lowest detectable concentration of h-IgA that can be distinguished from the zero standard is 0.5 µg/mL at the 95% confidence limit.

11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

h IgA	100.0 %
h IgA1	124.5 %
h IgA2	145.5 %
h IgG	<0.3 %
h IgM	<0.3 %

11.3. Correlation with RIA

The IBL IgA ELISA was compared to another commercially available IgA assay. 22 serum samples were analysed according in both test systems.

The linear regression curve was calculated

$$y = 1.5865x - 7.614$$

$$r = 0.9478 \quad (r^2 = 0.8984)$$

11.4. Hook Effect

The IgA ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 600 µg/mL

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

13. BIBLIOGRAPHY

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14. ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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