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Please use only the valid version of the Instructions for Use provided with the kit

Instructions for use

IgA Saliva ELISA

REF **SA E-6800R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

IgA Saliva ELISA

1 INTENDED USE

For Research Use.
Iga Saliva ELISA is a manual device intended for the quantitative determination of IgA in human saliva from an adult population.

2 PRINCIPLE OF THE METHOD

IgA Saliva ELISA is based on the simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on microwell plate and the other, polyclonal conjugated with horseradish peroxidase (HRP). After incubation the bound/free separation is performed by a simple solid-phase washing.
Then the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H_2SO_4) is added.
The colour intensity is proportional to the IgA concentration in the sample.
The IgA concentration in the sample is calculated through a standard curve.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. Standards

Cat. no.	Component	Standard	Volume/Vial
SA E-6801	**STANDARD A**	Standard A	1 ml
SA E-6802	**STANDARD B**	Standard B	1 ml
SA E-6803	**STANDARD C**	Standard C	1 ml
SA E-6804	**STANDARD D**	Standard D	1 ml
SA E-6805	**STANDARD E**	Standard E	1 ml
Content:	ProClin > 0.0015%		

2. SA E-6851 **CONTROL** **Control**
Content: ProClin > 0.0015%;
Concentration of Control is Lot-specific and is indicated on the QC-Report.
Volume: 1 x 1 ml

3. SA E-6813 **ASSAY-BUFF 5x** **Assay Buffer** (5X concentrated)
Content: Hepes buffer 25 mM pH 7.4; BSA 0.5 g/l; ProClin > 0.0015%
Volume: 1 x 40 ml

4. SA E-6840 **CONJUGATE-CONC** **Enzyme Conjugate** (20X concentrated)
Content: Antibody anti-IgA conjugated with horseradish peroxidase (HRP);
ProClin > 0.0015%; BSA 0.1%
Volume: 1 x 1 ml

5. SA E-6831 **96** **Coated Microtiterwells**
Content: 1 breakable microplate; Antibody anti-IgA adsorbed on microplate

6. MS E-0055 **SUBSTRATE** **Substrate Solution**
Content: H_2O_2-TMB 0.26 g/l (avoid any skin contact); ProClin < 0.0015%
Volume: 1 x 15 ml

7. MS E-0080 **STOP-SOLN** **Stop Solution**
Content: Sulphuric acid 0.15 mol/l (avoid any skin contact)
Volume: 1 x 15 ml

8. SA E-0030 **WASH-CONC 50x** **Wash Solution** (50X concentrated)
Content: NaCl 45 g/l; Tween-20 55 g/l; ProClin > 0.0015%
Volume: 1 x 20 ml

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3.2 Materials required but not provided

Distilled water

3.3 Auxiliary materials and instrumentation

Automatic dispenser
Precision Pipetting Devices
Microplate reader (450 nm, 620 – 630 nm)

4 WARNINGS

- This kit is intended for research use only.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- ⚠ Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents (standards, control, assay buffer, conjugate and wash solution) contain small amounts of ProClin™ 300 (> 0.0015%, < 0.06%) as preservative. Avoid contact with skin or mucosa.
- Classification according to Regulation (EC) No. 1272/2008 [CLP]
  Skin sensitivity, Category 1

  Warning

  - Contains: ProClin 300

  Hazard statements:
  H317 – May cause an allergic skin reaction.
  Precautionary statements:
  P261 – Avoid breathing dust/fume/gas/mist/vapours/spray.
  P280 – Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
  P321 – Specific treatment (see supplemental first aid instruction on this label).
  P333+P313 – If skin irritation or rash occurs: Get medical advice/attention.
  P362+P364 – Take off contaminated clothing and wash it before reuse.
- 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/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5 PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction for Use.
- All reagents should be stored refrigerated at 2 °C – 8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22 °C – 28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vial labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment the user has the responsibility to make sure that the kit has been appropriately validated for its intended use/purpose.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
  To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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 saliva.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Fresh disposable tips must be used when pipetting assay reagents including samples, standards and controls to mitigate the risk of carryover contamination. Failure to do so may lead to invalid results.

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6 REAGENT STORAGE AND STABILITY

Store the kit at 2 °C – 8 °C in the dark.

- The kit is stable at 2 °C – 8 °C until the expiry date stated on the external kit label.
- Once opened, the kit is stable at 2 °C – 8 °C for 6 months*.
- The diluted wash solution is stable for 30 days at 2 °C – 8 °C.
- The diluted assay buffer solution is stable for 30 days at 2 °C – 8 °C.
- The diluted conjugate solution is stable 3 hours at room temperature (22 °C – 28 °C).

* in use stability data supports reagent stability when used three times within this period.
Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

7 SAMPLE COLLECTION AND STORAGE

The assay should be performed using saliva samples.

Sample Storage	**Duration**
2 – 8 °C	24 hours
Freeze/thaw cycles	1 cycle
-20 °C	6 months

8 PROCEDURE

8.1 Preparation of the Standards and Control

Before use, mix for 5 minutes with a rotating mixer.
The standards are ready for use and have the following concentration:

	A	B	C	D	E
µg/ml	0	6.9	62	132	400

Note: The standard concentrations 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 Standards concentrations to be entered used for calculations are reported in the above table.
The control is ready to use; the concentration is printed on the label.

8.2 Preparation of the IgA Assay Buffer

Dilute the content of the vial **ASSAY-BUFF** **5x** with distilled or deionised water to a final volume of 200 ml prior to use. For smaller volumes respect the 1:5 dilution ratio.

8.3 Preparation of the Conjugate

Prepare immediately prior to use.
Add 50 µl of **CONJUGATE-CONC** to 950 µl of diluted IgA Assay Buffer (refer to section 8.2). The volume of diluted conjugate is proportional to the number of tests to be performed. Mix gently for 5 minutes with a rotating mixer.

8.4 Preparation of the Wash Solution

Dilute the content of each vial of the **WASH-CONC** **50x** with distilled water to a final volume of 1000 ml prior to use.
For smaller volumes respect the 1:50 dilution ratio.

8.5 Preparation of Samples

The determination of IgA can be performed in human saliva samples.
It is advised to collect the sample using a centrifuge glass tube and a plastic straw.
Do not use plastic tubes or commercially available devices for saliva collection to avoid false results.
Let the saliva flow down through the straw into the centrifuge tube; then centrifuge at 3000 rpm per 15 minutes.
Prepare the sample by diluting the supernatant liquid 1:20 with diluted assay buffer (e.g.: 50 µl supernatant + 950 µl diluted assay buffer); then mix gently for at least 5 minutes on a rotating shaker.
Further dilute this 1:50 with diluted assay buffer (e.g.: 20 µl supernatant + 980 µl diluted assay buffer).
Final dilution obtained: 1:1000.
Store the sample at -20 °C if the determination is not performed on the same day of the sample collection. Before using, mix gently, for 5 minutes, with a roller mixer.

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8.6 Procedure

Allow all reagents to reach room temperature (22 °C – 28 °C) for at least 30 minutes. At the end of the assay, immediately store the reagents at 2 °C – 8 °C: avoiding long exposure to room temperature.
Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2 °C – 8 °C.
To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the standard curve (Standard A – E), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample / Control	Blank
Standard A – E	25 µl		
Diluted Samples / Control		25 µl	
Diluted Conjugate	100 µl	100 µl	
Incubate 1 hour at room temperature (22 °C – 28 °C). Remove the contents from each well; wash the wells three times with 300 µl of diluted wash solution. **Important note:** during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer:** if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate Solution	100 µl	100 µl	100 µl
Incubate at 22 – 28 °C for 15 minutes in the dark.			
Stop Solution	100 µl	100 µl	100 µl
Shake gently the microplate. Read the absorbance (E) at 450 nm against a reference wavelength of 620 – 630 nm or against Blank within 5 minutes.			

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9 QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.
The kit control provided in the kit should be tested as unknown and is intended to assist in assessing the validity of results obtained with each assay plate.
The mean concentration of the control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.
The manufacturer recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.
When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[13].

10 CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean standard curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (preferred) or smoothed cubic spline curve fit, **including Standard A is required.** Other curve fitting algorithms are not recommended.
Alternatively, a standard curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Standard A should be included in the standard curve. Read the mean absorbance value of each unknown sample off the curve.

In order for the assay results to be considered valid the kit standards and control must fall within the specifications detailed in the lot specific certificate of analysis.
If a control is out of its specified range, the associated test results are invalid and samples must be retested.

11 MEASURING RANGE

The assay measuring range (AMR) is 3 – 400 µg/ml.
Any value that reads below 3 µg/ml should be reported as "< 3 µg/ml". Any value that reads above 400 µg/ml should be reported as "> 400 µg/ml".

12 METROLOGY AND TRACEABILITY

The IgA Saliva ELISA has been standardised against in-house reference standards (purified IgA in analyte-free buffered protein matrix).

13 UNCERTAINTY OF MEASUREMENT

The uncertainty associated with the measurement of the assay standards (A – E) was determined following CLSI EP29-A "Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline". The uncertainty for each standard level was assessed over a minimum of 5 days (1 run per day) by 3 different operators. Result from one representative lot:

Standard	Mean conc. (µg/ml)	Standard uncertainty Uc (µg/ml)
Standard A	< LoB	N/A
Standard B	7.0	0.54
Standard C	59.47	4.19
Standard D	140.34	18.21
Standard E	390.52	40.26

14 EXPECTED VALUES

Based on the literature data and on the results obtained with the IgA Saliva ELISA, the range of normality is:

	IgA saliva
Range of normality	40 – 170 µg/ml

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation.
The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own population.

15 PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

15.1 Detection Capability

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A2, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	µg/ml
Limit of Blank (LoB)	1.12 µg/ml
Limit of Detection (LoD)	2.3 µg/ml
Limit of Quantitation (LoQ)	3 µg/ml

15.2 Trueness

Trueness has been demonstrated through assessment of a serial dilution of a purified IgA standard prepared in-house.

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15.3 Precision

Precision of the IgA Saliva ELISA was determined by performing a complex precision study.
Repeatability: A total of 5 saliva samples were assayed in 5 replicates, once a day for 5 days by 3 operators. Data from 1 representative lot is shown below:

Sample	n	Mean Conc. (µg/ml)	Repeatability	
			SD	CV%
1	75	6.4	0.7	11%
2	75	39.1	1.9	5%
3	75	95.4	4.7	5%
4	75	134.4	9.9	7%
5	75	294.7	19.5	7%

Reproducibility: A total of 5 saliva samples were assayed in 5 replicates, once a day for 5 days by 3 operators. Data from 1 representative lot is shown below:

Sample	n	Mean Conc. (µg/ml)	Reproducibility	
			SD	CV%
1	75	6.4	0.9	15%
2	75	39.1	3.4	9%
3	75	95.4	7.5	8%
4	75	134.4	11.6	9%
5	75	294.7	29.4	10%

15.4 Linearity

Linearity was evaluated based on CLSI EP-06 Ed2, "Evaluation of the Linearity of Quantitative Measurement Procedures". A serial dilution of high and low saliva samples was prepared to create a panel from above the upper limit of linearity (ULL) to below the lower limit of quantitation (LLOQ).
For IgA concentration by IgA Saliva ELISA, the measurement procedure shows linearity for the interval from 3 to 407.33 µg/ml within the allowable deviation of linearity (ADL) of ± 15% or ± 3 µg/ml for concentrations below LoQ.

15.5 Analytical Specificity

The specificity was assessed with the following cross-reactants.

Cross-reactant	Concentration tested (unit)	Mean %Cross reactivity
IgA1	40 µg/ml	80.2%
IgA2	40 µg/ml	11.4%
IgG	4000 µg/ml	0.1%
IgM	4000 µg/ml	0.3%

The following substances do not interfere with a bias of > 15% in the IgA Saliva ELISA when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Albumin	1 g/dl
Citric acid	1%
Haemoglobin	200 mg/dl

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15.6 Hook effect

The hook effect was tested using concentrations of analyte up to 600 µg/ml. No hook effect was observed.

16 WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.
All materials that have come into contact with samples and reagents must be disposed of in accordance with country, state and local regulations.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!