DRG® IgA Salivary (SLV-4636)

CE

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RUO IN THE USA

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Immunoenzymatic colorimetric method for determination of IgA in saliva.

Salivary IgA ELISA is intended for laboratory use only.

2 PRINCIPLE

Salivary IgA ELISA is based on the simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on microwell plates 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 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 color 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. IgA **Standards** S0 – S4 (5 vials, 1 mL each)
2. IgA saliva **Control** (1 vial, 1 mL)
   Concentration of Control is Lot-specific and is indicated on Quality Control Report
3. 5X Conc. **IgA Assay Buffer** (1 vial, 40 mL)
   Hepes buffer 25 mM pH 7.4; BSA 0,5 g/L
4. 20X Conc. **Enzyme Conjugate** (1 vial, 1 mL)
   Antibody anti IgA conjugated with horseradish peroxidase (HRP)
5. Coated **Microtiterwells** (1 breakable microplate)
   Antibody anti IgA adsorbed on microplate
6. **Substrate Solution** (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L (avoid any skin contact)

7. **Stop Solution** (1 vial, 15 mL)
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
8. 50X Conc. **Wash Solution** (1 vial, 20 mL)
   NaCl 45 g/L; Tween-20 55 g/L

3.2 Reagents necessary not supplied

Distilled water

3.3 Auxiliary materials and instrumentation

Automatic dispenser

Microplates reader (450 nm)

Note

Store all reagents between 2 °C - 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.

4 WARNINGS

- This kit is intended for use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300® 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/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of IgA from 0.5 µg/mL to 400 µg/mL.

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 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, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 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.

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 Standard concentrations to be entered in the instruments for calculation are:

	S0	S1	S2	S3	S4
µg/mL	0	6,9	62	132	400

Once opened, the standards are stable 6 months at 2 °C - 8 °C.

6.2 Preparation of IgA Assay Buffer

Dilute the content 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 diluition ratio 1:5.

Store at 2 °C - 8 °C until the expiry date printed on the label.

6.3 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 °C - 28 °C).

6.4 Preparation of Wash solution

Dilute the content of each vial of the *50X Conc. Wash Solution* with distilled water to a final volume of 1000 mL prior to use.

For smaller volumes respect the 1:50 dilution ratio.

The diluted wash solution is stable for 30 days at 2 °C - 8 °C.

6.5 Preparation of the Sample

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

Let the saliva flow down trough 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 *dilution A* for each sample by diluting supernatant liquid 1:20 with diluted Assay Buffer (e.g.: 50 µL + 950 µL); then mix gently every dilution A by leaving it for at least 5 minutes on a rotating shaker and dilute this *dilution A* 1:50 with diluted Assay Buffer (e.g.: 20 µL + 980 µL).

Final 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.

6.6 Procedure

Allow all reagents to reach room temperature (22 °C - 28 °C).

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 calibration curve (S0-S4), two for each Control, two for each sample, 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 °C - 28 °C). Remove the contents from each well; wash the wells three times with 300 µL of diluted wash solution.			
TMB Substrate Solution	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22 °C - 28 °C).			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7 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. Fresh reagents should be used to determine the reason for the variations.

8 RESULTS

8.1 Mean Absorbance

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

8.2 Calculation of Results – Automatic method

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

8.3 Calculation of Results – Manual method

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

1. Record the absorbance obtained from the printout of the microplate reader.
2. Plot the absorbance for each duplicate serum reference versus the corresponding IgA concentration in µg/mL on linear graph paper.
3. Connect the point with a best-fit curve.
4. 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). 600 µg/mL

9 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

10 BIBLIOGRAPHY

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11 TROUBLESHOOTING

POSSIBLE ERROR 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 (CV%)

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

Too high between-run (CV%)

- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation