DRG® IgA Salivary (SLV-4636)

CE

REVISED 22 NOV. 2010 RM (VERS. 2.1)

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1 INTENDED USE

Immunoenzymatic colorimetric method for determination of h-IgA in saliva.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

2 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 (HPR). After incubation the bound/free separation is performed by a simple solid-phase washing, then the substrate solution (TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance is determinated.

The h-IgA concentration in the sample is calculated based on a series of standard. The color intensity is proportional to the h-IgA concentration in the sample.

3 REAGENT, MATERIAL AND INSTRUMENTATION

3.1 Reagent and material supplied in the kit

1. IgA **Standards S0 – S4**; 5x (1 vial = 1 mL)
2. IgA. **Assay Buffer Conc** (5x) (1 bottle) 40 ml
   Hepes buffer 25 mM pH 7.4; BSA 0.5 g/L
3. **Enzyme Conjugate Conc.** (1 vial) 0.7 mL
   Conjugated anti-IgA
4. Coated **Microplate** (1 microplate breakable)
   Anti-IgA adsorbed on microplate
5. TMB **Substrate Solution** (1 bottle) 12 mL
   H_2O_2-TMB 0.25g/L (avoid any skin contact)
6. **Stop solution** (1 bottle) 12 mL
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
7. **Wash Solution Conc.** (50x) (1 bottle) 20 mL
   NaCl 9 g/L, Tween 1 g/L, Proclin 300 0.1 mL/L

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser. Microplates reader

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Note
Store all reagents between 2°C - 8C° in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use. Do not remove the adhesive sheets on the strips unutilized.

4 PRECAUTION

- The reagents contain Proclin 300 as preservative.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Use only reagents supplied in the kit.
- This method allows the determination of IgA from 0.5 µg/mL to 400 µg/mL.

5 PROCEDURE

5.1 Preparation of the Standard (S0, S1, S2, S3, S4)

The standards have approximately the following concentration: 0; 6.9; 62; 132, 400 **ng/mL**.

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

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

The concentrations to be entered in the instruments for calculations are:

	S0	S1	S2	S3	S4
µg/mL	0	6.9	62	132	400

5.2 Preparation of IgA Assay Buffer

Dilute contents of IgA Assay Buffer Conc. with 160 mL of distilled or deionized water in a suitable storage container.

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

5.3 Preparation of Enzyme Conjugate

Prepare immediately before use.

Add 50 µl stock solution (reagent 3) to 1.0 mL of diluted IgA Assay Buffer (reagent 2).

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.

5.4 Preparation of Wash solution

Dilute contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container.

Store at room temperature 20°C - 27°C for up to 60 days.

5.5 Preparation of the Sample

For sample collection it 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.

Dilution A (1:20):
50 µL liquid sample supernatant + 950 µL diluted Assay Buffer;
Mix gently by leaving it for at least 5 minutes on a rotating shaker

Dilution B (1:50):
20 µL Dilution A + 980 µL diluted Assay Buffer
Mix gently by leaving it for at least 5 minutes on a rotating shaker.

The finally dilution obtained is 1:1000.

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

5.6 PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S0-S4), two for each sample, one for Blank.

Pipette:

	Standard	Sample	Blank
Diluted sample	---	25 µL	---
Standard S_0–S_4	25 µL	---	---
Diluted Conjugate	100 µL	100 µL	---

Incubate at room temperature (22°C - 28°C) for *1 hour*.

Remove the contents from each well; wash the wells with 300 µL of diluted wash solution. Repeat for three times the washing procedure by draining the water completely.

Pipette:

	Standard	Sample	Blank
TMB-Substrate	100 µL	100 µL	100 µL

Incubate at room temperature (22°C - 28°C) for 15 minutes in the dark.

Pipette:

	Standard	Sample	Blank
Stop Solution	100 µL	100 µL	100 µL

Read the absorbance (E) at 450 nm against Blank.

6 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.

7 BIBLIOGRAPHY

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8 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