DRG® Alpha Amylase Saliva (ENZ-5127)

Revised 24 Nov. 2010 rm (Vers. 1.1) **USA: RUO**

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

INTENDED USE

The α-Amylase Saliva assay is a kinetic colorimetric method for determination of α-amylase in saliva.

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

PRINCIPLE

The human α-Amylase hydrolyses the 2-chloro-4 nitrophenyl-α-maltotrioside (CNP-G3) in glucose polymers and short-chain p-nitrophenyl-oligosaccharide with formation of 2-chloro-4-nitrophenol (CNP).

The increase of the extinction is evaluated spectrophotometrically at 405 nm and is proportional to α-amylase activity in the sample.

REAGENT, MATERIAL AND INSTRUMENTATION

1.1 Reagent and material supplied in the kit

1. **Reagent A** (1 bottle) 32 mL
   CNP-G3 2 mmol/L, Goods buffer 100 mmol/L,
   stabilisers and preservatives
2. **Conc. Assay Buffer 5x** (1 bottle) 50 mL
   Hepes buffer 200 mM pH 7.4; BSA 0,5 g/L
3. **Microplate**

1.2 Reagents necessary not supplied

Distilled water.

1.3 Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (405 nm).

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

PRECAUTION

- Once open, store all reagents between 2°C – 8°C and do not use them beyond the expiration date.
- This kit is intended for Research Use Only. Not for use in diagnostic procedures.
- Avoid the contact with reagents which could be toxic if are ingested. Not pipette with the mouth.

PROCEDURE

1.4 Preparation of Assay Buffer

Dilute the whole bottle of *Assay Buffer Conc 5x* in 200 mL of distilled or deionized water.

Keep between 2°C – 8°C until expiration date (see vial label).

1.5 Preparation of the Sample

For sample collection it is advised to use glass centrifuge tubes and plastic straws.

Do not use plastic tube or commercially available devices for the saliva collection to avoid false results.

Let the saliva flow down through the straw into the centrifuge glass tube, freeze and thaw it to help to mucins precipitation. Centrifuge at 3000 rpm for 15 minutes.

Dilute 10 µL of liquid supernatant to 1 mL of diluted Assay Buffer (reagent 2).

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 samples at -20°C.

1.6 PROCEDURE

All the reagents should be brought to room temperature 22°C - 28°C.

If you should manually dispense a high number of samples, it is advised to use maximum four strips for each tests.

Format the microplate wells for each specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal off and store at 2°C – 8°C

	Sample	Blank
Diluted Sample	10 µL	---
Distilled water	---	10 µL
Reagent A	300 µL	300 µL

Incubate at 37°C for *3 min* immediately after reagents dispensation.

At the end of incubation time put the microplate on microplate reader at ambient temperature (22-28°C) and read the **absorbance variation (ΔA)** two times at 405 nm, the first after 1 minute and the second after 5 minute from the end of incubation time, subtracting each time the absorbance of blank.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of α-amylase 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.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES / LITERATURE

1. Tietz NW. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia: WB Saunders Company: 46-49 (1995)
2. (IFCC) "Approved Recommendation on IFCC Methods for the measurement of catalytic concentration of Enzymes" _Part 9
3. Clin Chem Lab Med 36(3): 185 –203 (1998)

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TROUBLESHOOTING

ERRORS / POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no Reagent A pipetted
- contamination of Reagent A
- 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 Reagent A (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect Reagent A (e.g. not from original kit)
- incubation time too long, incubation temperature too high

Unexplainable outliers

- contamination of pipettes, tips or containers

too high within-run CV%

- reagents and/or strips not pre-warmed to room temperature prior to use
- incubation conditions not constant (time, temperature)

too high between-run CV %

- too long dispense time
- person-related variation

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