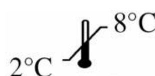
DCM075-3
Ed. 03/2012

α-AMYLASE SALIVA

Enzymatic determination of α-AMILASE in saliva.

RUO LOT See external label 

Σ = 96 test REF DKO075

INTENDED USE

Eagle Bioscience α-Amylase Saliva Assay Kit is a kinetic colorimetric method for quantitative determination of α-amylase in saliva. The kit is for research use only and not to be used for diagnostic procedures.

1. CLINICAL SIGNIFICANCE

Amylase is the name given to enzymes that break down starch. They are classified as saccharidases, enzymes that cleave polysaccharides.Although the amylases are designated by different greek letters, they all act on α-1,4 glycosidic bonds. The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. The α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. In animals, it is a major digestive enzyme.

Although found in many tissues, the α-amylase is most prominent in pancreatic juice and saliva which each have their own isoform. Salivary α-amylase breaks starch down into maltose and dextrin. This form is also called ptyalin. Ptyalin will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, achrodextrin) producing successively smaller starches and ultimately maltose. Ptyalin acts on linear α(1,4) glucosidic linkages, but compound hydrolysis requires an enzyme which acts on branched products. Salivary amylase is inactivated in the stomach by gastric acid. Pancreatic α-amylase randomly cleaves the α(1-4)glycosidic linkages of amylose to yield dextrin, maltose or glucose molecules.

2. PRINCIPLE

In the α-Amylase Saliva Assay Kit, 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.

3. REAGENT, MATERIAL AND INSTRUMENTATION

3.1. Reagent and material supplied in the kit

1. Reagent A (1 bottle, 32 mL)
CNP-G3 2mmol/L, Goods buffer 100 mmol/L, stabilisers and preservatives **REF DCE042-0**
2. Conc. Assay Buffer 5x (1 bottle) 50 mL
Hepes buffer 200 mM pH 7.4; BSA 0,5 g/L **REF DCE049/7549-1**
3. Microplate **REF DMZ001-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (405 nm).

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

4. PRECAUTION

- Once open, store all α-Amylase Saliva Assay Kit reagents between 2-8°C and do not use them beyond the expiration date.
- Avoid the contact with α-Amylase Saliva Assay Kit reagents which could be toxic if are ingested. Not pipette with the mouth.

5. PROCEDURE

5.1. Preparation of Assay Buffer

Dilute the whole bottle of Assay Buffer Conc 5x in 200 mL of distilled or deionized water. Keep between 2-8°c until expiration date (see vial label).

5.2. Preparation of the Sample

For sample collection 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 supernatant liquid 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.

5.3. Procedure

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

If you should manually dispense a high number of samples, it is advised to use to the 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÷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.

6. 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.

7. LIMITATION OF PROCEDURE

7.1. Assay Performance

It is important that the time of reaction in each well is held constant for reproducible results. Plate readers measure the OD vertically. Do not touch the bottom of the wells.

8. RESULTS

Calculate il $\Delta A/Min. = [(A_{5\ min} - A_{1\ min}) / 4]$

Calcul: α-Amylase (U/mL):

$$\frac{\Delta A/min \times TV \times DF}{MMA \times SV \times LP}$$

Where :

ΔA/Min = Absorbance difference per minute

TV = Total assay volume (0.310 mL)

DF = Dilution factor

MMA = Millimolar absorption (extinction) coefficient of 2-chloro-p-nitrophenol (12.9)

SV = Sample volume (0.010 mL)

LP = Light path in centimeters = 0.95 (specific to microplate received with kit)

$$\frac{\Delta A/min \times 0.310 \times 100}{12.9 \times 0.010 \times 0.95} = \Delta A/min \times 253 = U/mL^{(*)}$$

$^{(*)}$ = α-amylase activity in sample

Note:

The microplate provided with the kit is not chemically treated and its use is intended like a batch of reaction. Otherwise the reaction may be conduct in a different batch (for example in a spectrophotometer cuvette) and it is important to know the right optical path lenght LP that is the distance the light travels through the sample; for standard cuvette is generally 1 cm.

9. REFERENCE VALUE

Adults (Normal)	80.0 U/mL
Absolute Range:	4 – 400 U/mL

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra-Assay

Within run variation was determined by replicate determination (16x) of three different levels of control sera in one assay. The within assay variability is < 1.5 %.

10.1.2. Inter-Assay

Between run variation was determined by replicate measurements of three different saliva samples in 2 different lots. The between assay variability is <1.5%.

10.2. Sensitivity

The lowest detectable concentration of α-amylase that can be distinguished from the zero standard is 2.5 U/mL at the 95 % confidence limit.

10.3. Correlation

The α-Amylase kit (Diametra) was compared to a similar commercially available kit.
The linear regression curve was calculated
$y = 1.19\,x + 2.55$
$r = 0.998$ ($r^2 = 0.996$)

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES

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

Ed 03/2012 **DCM075-3**

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ERROR 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 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, CV% too high within-run
- reagents and/or strips not pre-warmed to room temperature prior to use
- incubation conditions not constant (time, temperature), CV % too high between-run
- too long dispense time
- person-related variation