EN

# Alpha-Amylase

CODE **984373**

| | |
|---|---|
| ❶ | 6 x 20 ml Reagent A |
| ❷ | 2 x 5 ml Reagent B |

## INTENDED USE

Reagent for photometric determination of Alpha-Amylase in homogenous liquid samples using automated Thermo Scientific™ Gallery™ or Arena™ analyzers.

## METHOD

Enzymatic test with 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7) (equals to ethylidene-pNP-G7 (E-pNP-G7)) as the substrate.
Method is performed at 37 °C, using 405 nm filter.

## PRINCIPLE OF THE PROCEDURE

Substrate is cleved by the α-amylase activity from the sample. The produced fragments are hydrolyzed by α-glucosidase, which causes the release of the chromophore. The activity of α-amylase can be determined by the rate of increase in absorbance at 405 nm as p-nitrophenol (PNP) is produced. The use of ethylidene prevents exo-enzymes from breaking down the substrate, so in the absence of α-amylase, no colour change is observed.

$$5\ \text{EPS-G7} + 5\ H_2O \xrightarrow{\alpha\text{-Amylase}} 2\ \text{Ethylidene-G5} + 2\ \text{G2PNP} + 2\ \text{Ethylidene-G4} + 2\ \text{G3PNP} + \text{Ethylidene-G3} + \text{G4PNP}$$

$$2\ \text{G2PNP} + 2\ \text{G3PNP} + \text{G4PNP} + 14\ H_2O \xrightarrow{\alpha\text{-Glucosidase}} 5\ \text{PNP} + 14\ \text{G}$$

(PNP = p-nitrophenol, G = glucose)

## REAGENT INFORMATION

For this method two reagents, Reagent A and Reagent B, are needed.

| **Ready-to-use reagents** | | **Barcode id** |
|---|---|---|
| Reagent A | 6 x 20 ml | A68 |
| Reagent B | 2 x 5 ml | A69 |

**Note:** Labels of reagents vials have two barcodes.
For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.
For Arena analyzers, turn the short barcode on the left side to the barcode reader.

**Concentrations/compositions**

| | | |
|---|---|---|
| **Reagent A** | | |
| | Good's buffer, pH 7.2 ± 0.1 | 0.1 mol/l |
| | α-Glucosidase | ≥ 2000 U/l |
| | NaCl | 62.5 mmol/l |
| | $MgCl_2$ | 12.5 mmol/l |
| | $NaN_3$ | < 0.1% |
| **Reagent B** | | |
| | Good's buffer, pH 7.2 ± 0.1 | 0.1 mol/l |
| | EPS-G7 | 8.5 mmol/l |
| | $NaN_3$ | < 0.1% |

**Precautions**
The reagents contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.
Take the necessary precautions for the use of laboratory reagents. Prevent contamination by salivary and sweat amylase.

**Preparation**
The reagents A and B are ready-to-use.
**Note:** Check that there are no bubbles in the bottle neck or on the surface of the reagent when you insert vials in the analyzer.

**Storage and Stability**
Reagents in unopened vials are stable at 2…8 °C until the expiry date printed on the label. Do not freeze! Protect the reagents from light. Avoid contamination with saliva.
The on board stability of reagents should be validated by the user according to used application and sample matrix. The on board stability stated in the Application Proposal Notes has been determined using serum alpha-amylase as a sample.
The shelf-life of the product has been validated using serum alpha-amylase as a sample.

## SAMPLES

**Sample Type**
Homogenous liquid samples.

**Note:** Suitability of the sample material must be validated by the user. Method and application is originally intended for determination of human serum alpha-amylase.

**Sample concentration and Gallery/Arena application**
All method related details are in the separate application proposal note.

If the Gallery or Arena applications have a primary dilution of 1+4, this means that every sample is automatically first diluted with 1+4 (equals to 1:5 dilution).

## TEST PROCEDURE

See a separate application proposal for the Gallery or Arena analyzers.

**Materials required but not provided**
Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

**Calibration**
Calibration should be perfomed by using the same alpha-amylase as in the samples to be measured.

**Quality Control**
Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.
If the same standard material is used for calibration and quality control, an additional internal control is recommended to be used.

## CALCULATION OF RESULTS

The results are calculated automatically by the analyzer using a calibration curve.

## LIMITATIONS OF THE PROCEDURE

**Interference**
Possible interfering analytes in the sample matrix should be tested by the user.

## MEASURING RANGE

The test has been developed using human serum Alpha-Amylase activity within a measuring range from 10 to 1500 U/l. Extended measuring range after secondary dilution: 10 - 7500 U/l.
Measuring range for other matrices should be validated by the user.

## PERFORMANCE CHARACTERISTICS

Each laboratory is responsible to verify the method to prove the analysis performance. E.g. sample matrix, concentrations or analysis environment may effect the results obtained.

## WASTE MANAGEMENT

Please refer to local legal requirements. It is recommended to empty the cuvette waste bin and waste water container daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
**Note:** If using reagents/solutions that react with each other, cuvette waste bin and waste water container should be emptied and washed between use of these reagents.



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**OTHER REMARKS**
Each laboratory is responsible to verify the method to prove the analysis performance. All results must be verified by laboratory quality control samples.
Manufacturer does not warrant that the product is error-free or will accomplish any particular result. In no event shall the manufacturer be liable for special, incidental, indirect, punitive or consequential damages (including, but not limited to, loss of profits, loss of goodwill, loss of data or loss of use damages) arising out of the use or disposition of the products.

**REFERENCE FOR METHOD PRINCIPLE**
AACC Method 22-02.01
AOAC Official Method 2002.01

**ADDITIONAL MATERIAL**
Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Application proposals for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

**MANUFACTURER**

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**Changes from previous version**
New Instructions for Use.



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