Technical Bulletin

α-Amylase Activity Assay Kit

Catalogue Number MAK478

Product Description

Amylase belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α-1,4-glycosidic bonds. The α-amylases cleave at random locations on the starch chain, ultimately yielding maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme. Increased enzyme levels in humans are associated with salivary trauma, mumps due to inflammation of the salivary glands, pancreatitis, and renal failure.

Simple, direct, and automation-ready procedures for measuring amylase activity are useful. The α-Amylase Activity Assay Kit method involves two steps. First, α-amylase in the sample hydrolyses starch and the product is rapidly converted to glucose by α-glucosidase and hydrogen peroxide by glucose oxidase. Next, the hydrogen peroxide concentration is determined with a colorimetric reagent at 585 nm.

The linear detection range of the kit is 0.3 – 50 units/liter (U/L) α-amylase. The kit is suitable for the determination of α-amylase activity in blood, saliva, urine, grains, and other agricultural samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer (pH 7.0) 20 mL
  Catalogue Number MAK478A
- Substrate 120 µL
  Catalogue Number MAK478B
- Detection Reagent 20 mL
  Catalogue Number MAK478C
- Enzyme A 120 µL
  Catalogue Number MAK478D
- Enzyme B 120 µL
  Catalogue Number MAK478E
- Glucose Standard (300 mg/dL) 1 mL
  Catalogue Number MAK478F

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

For samples containing glucose only:

- Microcon-10kDa Centrifugal Filter Unit with Ultracel-10 membrane
  (Catalogue Number MRCPRT010 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 × *g*

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme A and Enzyme B: Keep thawed enzymes on ice during the assay.

Substrate: The substrate may have precipitates. Prior to use, vortex the tube to dissolve precipitates.

Detection Reagent: Gently swirl the Detection Reagent bottle prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Ideally samples are assayed fresh. When stored frozen, α-amylase is stable for one month. Ascorbic acid, heparin, EDTA, EGTA, citrate, SDS, Tris (>8 mM) and ethanol (>0.4%) interfere with the assay and should be avoided in sample preparation.

For unknown Samples, perform a pilot experiment by testing several dilutions to ensure the readings are within the linear detection range of the kit.

Recommended dilutions: serum 50-fold, saliva 2,000-fold. Perform dilutions in Assay Buffer prior to assay.

Add 10 µL of each Sample into separate wells of a clear flat-bottom 96-well plate.

Samples Containing Glucose

1. For samples known to contain glucose, use a membrane filter (such as, Micron-10kDa Centrifugal Filter Unit with Ultracel-10 membrane) to remove the glucose.
2. Load 50 µL of Sample into the membrane filter.
3. Add 500 µL of Assay Buffer.
4. Centrifuge at 14,000 × *g* for 30 minutes at room temperature.
5. Check the level of sample in the membrane filter. Ideally the sample level will be less than 50 µL.
6. Add 500 µL of Assay Buffer and repeat the centrifugation.
7. Measure the final sample volume with a calibrated pipette.
8. Calculate the dilution factor (DF). DF = Final sample volume/50 µL.
9. Add 10 µL of each prepared Sample into separate wells of a clear flat-bottom 96-well plate.

400 µM Glucose Standard

Prepare a 400 µM Glucose Standard by mixing 10 µL of the 300 mg/dL Glucose Standard with 406 µL of Assay Buffer. Mix well. Transfer 10 µL of the 400 µM Glucose Standard into separate wells of the plate.

Blank

Transfer 10 µL of Assay Buffer into separate wells of the plate.

Working Reagent

Note: This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 42.5 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	40 µL
Substrate	0.5 µL
Enzyme A	1 µL
Enzyme B	1 µL

2. Transfer 40 µL of Working Reagent into each well. Tap plate to mix.

Detection and Measurement

1. Incubate the plate for 15 minutes at 25 °C.
2. Add 150 µL of Detection Reagent to each well and mix.
3. Incubate the plate for 20 minutes at 25 °C.
4. Read optical density (OD) at 585 nm.

Results

Calculate the α-Amylase activity of Sample.

α-Amylase (U/L) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{400}{T\ (min)} x\ DF$$

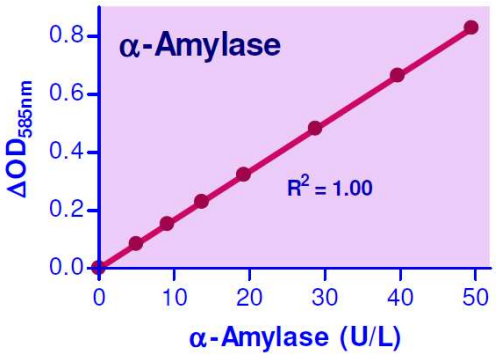
where:

OD_{Sample} =	Optical density reading of Sample
OD_{Blank} =	Optical density reading of Blank
$OD_{Standard}$ =	Optical density reading of Standard
400 =	Concentration of the standard in mg/dL
T =	Incubation time in minutes
DF =	Sample dilution factor (for example, DF = 50 for serum and 2000 for saliva)

If the calculated activity is higher than 50 U/L, dilute Sample in Assay Buffer and repeat the assay. Multiply the results by the dilution factor (DF).

Unit Definition: One unit of α-amylase catalyzes the production of 1 µmole of glucose per minute under the assay conditions.

Typical α-Amylase Activity Standard Curve



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