α-Amylase Assay Kit

Rapid Colorimetric Determination of α-Amylase Activity at 595nm

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 (EC 3.2.1.1) 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 α-amylase activity are very desirable. amsbio's α-amylase assay uses an insoluble dye-coupled substrate amylose azure, which is cleaved by α-amylase into soluble colored products. The color intensity, measured at 595 nm, is proportionate to the enzyme activity in the sample.

APPLICATIONS

Direct assays of α-amylase activity in serum, heparinized plasma, saliva, urine and other biological samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 2 to 300 U/L α-amylase in 96-well plate assay.
Convenient. The procedure involves adding a single working reagent, and reading the optical density at 5 min at room temperature or 37°C.

KIT CONTENTS

Substrate (pH 7.0): 20 mL
Stop Reagent: 10 mL
Calibrator: 2 mL (equivalent to 550 U/L)

Storage conditions. Store all reagents at 2-8°C. Shelf life of at least 6 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample preparation. Ideally samples are assayed fresh. When stored frozen, α-amylase is stable for one month. EDTA, EGTA and citrate are α-amylase inhibitors and should be avoided in sample preparation.

1. *Reaction*. In appropriately labeled 1.5-mL Eppendorf tubes, transfer 10 µL sample. Add 190 µL Substrate, vortex briefly to mix and incubate for 5 min.

   Caution: the substrate contains blue insoluble materials that tend to settle. It is important to invert the bottle ten times to maintain the substrate in suspension and then quickly transfer the substrate into sample tubes. If the assay is to be performed at 37°C, warm up the Substrate to this temperature prior to sample addition.

   As a blank control, use 10 µL water plus 190 µL Substrate and 80 µL Stop Reagent. Alternatively a blank control can be prepared in the order: 10 µL sample, 80 µL Stop Reagent and 190 µL Substrate. This would be necessary if the sample has a background color that is visibly blue.

2. Add 80 µL Stop Reagent to each sample tube to terminate the reaction. Vortex to mix and centrifuge for 5 min at 14,000 rpm. Carefully transfer 200 µL supernatant into wells of a clear bottom 96-well plate.

   In separate wells, transfer 200 µL water and 200 µL Calibrator.

3. Read OD595nm (580 to 600nm) on a plate reader. Signal is stable for at least one hour.

4. *Calculation:* α-amylase activity is calculated as follows,

$$\text{Activity} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{CAL} - OD_{H2O}} \times n \times 550 \text{ (U/L)}$$

where OD_{SAMPLE} and OD_{BLANK} are the OD_{595nm} values of the sample and blank, respectively. OD_{CAL} and OD_{H2O} are the OD_{595nm} values of the Calibrator and water. *n* is the dilution factor. The number "550" is the equivalent activity (U/L) of the calibrator under the assay conditions.

Note: if the calculated activity is higher than 300 U/L, dilute sample in water and repeat assay. Multiply the results by the dilution factor (*n*).

Unit definition: one unit of enzyme catalyzes the production of 1 µmole of product per minute under the assay conditions (pH 7.0).

MATERIALS REQUIRED, BUT NOT PROVIDED

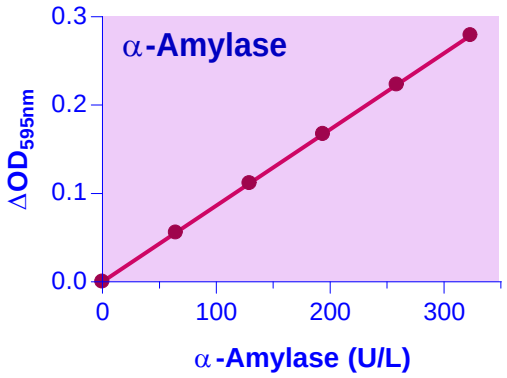
Pipeting (multi-channel) devices. 1.5-mL Eppendorf tubes. Centrifuge. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERATIONS

For assays in standard 1 mL cuvet, use 1 mL water and 1 mL Calibrator. Sample reactions: 40 µL sample + 720 µL Substrate and 320 µL Stop Reagent, after incubation and centrifugation, remove 1 mL for OD determination.

EXAMPLES

Samples were assayed in duplicate using the 96-well plate protocol. α-amylase activities were 149 ± 3 U/L for rat plasma, 172 ± 6 U/L for rat serum, 164 ± 2 U/L for mouse serum, 28.4 ± 0.8 U/L for human serum, 29.9 ± 0.4 U/L for human plasma, 27.9 ± 0.4 U/L for human urine and 10,578 ± 390 U/L for a human saliva sample.



LITERATURE

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