EnzyChrom™ α-Amylase Assay Kit (ECAM-100)

Quantitative Colorimetric Amylase Determination at 585nm

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 amylase activity are very desirable. BioAssay Systems' EnzyChrom™ α-amylase assay method involves two steps: (1). α-amylase in the sample hydrolyzes starch and the product is rapidly converted to glucose by α-glucosidase and hydrogen peroxide by glucose oxidase; (2). hydrogen peroxide concentration is determined with a colorimetric reagent.

APPLICATIONS

Determination of α-amylase activity in blood, saliva, urine, grains and other agricultural samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.3 to 50 U/L α-amylase in 96-well plate assay.
Convenient. The procedure involves adding a single working reagent, incubation for 15 min, followed by the detection reagent and a 20-min incubation and reading the optical density at 585 nm.

KIT CONTENTS

Assay Buffer (pH 7.0): 20 mL	**Substrate: 120 µL**
Detection Reagent: 20 mL	**Enzyme A: 120 µL**
Glucose Standard: 1 mL	**Enzyme B: 120 µL**

Storage conditions. Kit is shipped on ice. Store all components at -20°C. Shelf life: 6 months after receipt.

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

Reagents. Equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice. The substrate may have precipitates. Prior to use, vortex tube to dissolve precipitates; gentle swirl the Detection Reagent bottle.

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 (> 8mM) and ethanol (>0.4%) interfere and should be avoided in sample preparation. If glucose is present in the sample, treat the samples as described in GENERAL CONSIDERATIONS. It is prudent to perform a pilot test with samples at various dilutions. Recommended dilution: serum 50-fold, saliva 2,000-fold in Assay Buffer prior to assay.

1. Prepare 400 µM Glucose Standard by mixing 10 µL of the provided (300 mg/dL) standard with 406 µL Assay Buffer. Transfer 10 µL Assay Buffer, 10 µL 400 µM glucose, and 10 µL of each sample into separate wells of a clear flat-bottom 96-well plate.
2. Prepare enough Working Reagent for each well by mixing 40 µL Assay Buffer, 0.5 µL Substrate, 1 µL Enzyme A, 1 µL Enzyme B.

   Transfer 40 µL Working Reagent to each well. Incubate for 15 min at room temperature (25°C).
3. Add 150 µL Detection Reagent to each well. Mix and incubate for 20 min at room temperature (25°C). Read OD585nm (540-610nm) on a plate reader.

CALCULATION

The Amylase activity is calculated as

$$\text{Activity} = \frac{OD_{SAMPLE} - OD_{BUFFER}}{OD_{STD} - OD_{BUFFER}} \times \frac{400}{t\,(\text{min})} \times n \quad (\text{U/L})$$

OD_{SAMPLE}, OD_{STD} and OD_{BUFFER} are optical density values of the sample, the 400 µM glucose standard and Assay Buffer. t is the incubation time. t = 15 min in the standard protocol. n is the dilution factor (n = 50 for serum, 2000 for saliva). One unit of enzyme catalyzes the production of 1 µmole of glucose per min under the assay conditions.

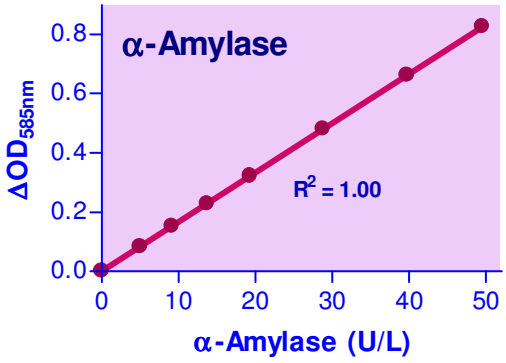
Note: if the calculated activity is higher than 50 U/L, dilute sample in Assay Buffer and repeat assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, clear flat-bottom 96-well plates, plate reader, and optionally membrane filters (e.g. Microcon YM-10 from Millipore).

GENERAL CONSIDERARIONS

For samples known to contain glucose, use a membrane filter (e.g. Microcon YM-10 from Millipore) to remove glucose: load 50 µL sample in a Microcon YM-10 (10 kDa cutoff) and add 500 µL Assay Buffer. Centrifuge at 14000 rpm for 30 min, check level of sample, ideally the sample level will be less than 50 µL. Add 500 µL Assay Buffer and repeat the centrifugation. Measure final sample volume with a pipetman and calculate dilution factor n = final sample volume/50 µL.



Standard Curve in 96-well plate assay

PUBLICATIONS

1. Han, M. J. (2020). Novel bacterial surface display system based on the Escherichia coli protein mipa. Journal of Microbiology and Biotechnology. 30(7): 1097-1103.

2. Bae, G.-S. (2020). Protective effect of nypa fruticans wurmb. Water extract on acute pancreatitis. Journal of Physiology & Pathology in Korean Medicine. 34(6): 334-340.

3. Lee, Sang-Bum, et al (2019). Impacts of whey protein on starch digestion in rumen and small intestine of steers. Journal of Animal Science and Technology 61.2: 98-108.

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