

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

Direct assays 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 μ L
Detection Reagent: 20 mL	Enzyme A: 220 μ L
Glucose Standard: 1 mL	Enzyme B: 220 μ L

Storage conditions. Kit is shipped on dry ice. Store Substrate and Detection Reagent at 4°C and others at -20°C. Shelf life of at least 6 months (see expiry dates on labels).

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.

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. 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 μ M Glucose Standard by mixing 10 μ L of the provided (300 mg/dL) standard with 406 μ L Assay Buffer. Transfer 10 μ L Assay Buffer, 10 μ L 400 μ M glucose, and 10 μ L 2 x sample (one sample and one sample blank) into separate wells of a clear flat-bottom 96-well plate.
2. Prepare an appropriate Working Reagent for each well. For Standards and samples: mix 40 μ L Assay Buffer, 0.5 μ L Substrate, 2 μ L Enzyme A, 2 μ L Enzyme B. For the sample blanks, 42 μ L Assay Buffer, 0.5 μ L Substrate, 2 μ L Enzyme B. This sample blank (OD_{BLANK}) allows the quantification of free glucose in the sample.

Transfer 40 μ L working reagent and blank control reagent to the appropriate sample and blank wells. Tap plate to mix. Incubate for 15 min at room temperature.

3. Add 150 μ L Detection Reagent to each well. Mix and incubate for 20 min at room temperature. Read OD_{585nm} (540-610nm) on a plate reader.

CALCULATION

The Amylase activity is calculated as

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

OD_{SAMPLE}, OD_{BLANK}, OD_{STD} and OD_{BUFFER} are optical density values of the sample, sample blank, the 400 μ M 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 μ mole of glucose per min under the assay conditions.

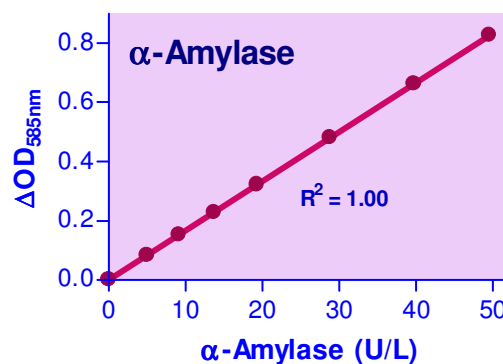
Note: if the calculated activity is higher than 50 U/L, dilute sample in Assay Buffer and repeat assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERARIONS

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



Standard Curve in 96-well plate assay

LITERATURE

1. Gullbault, GG and Rietz, E.B. (1976). Enzymatic, Fluorometric Assay of α -Amylase in Serum. Clin. Chem. 22(10): 1702:1704.
2. Mashige F, Ohkubo A, Kamei S, Yamanaka M. (1982). An enzymic method for urine and serum alpha-amylase assay. Clin. Chem. 28(7):1715-6.
3. Marshall JJ, Iodice AP, Whelan WJ. (1977). A new serum alpha-amylase assay of high sensitivity. Clin Chim Acta. 76(2):277-83.