

## EnzyChrom™ $\alpha$ -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  $\alpha$ -1,4-glycosidic bonds. The  $\alpha$ -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,  $\alpha$ -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™  $\alpha$ -amylase assay method involves two steps: (1)  $\alpha$ -amylase in the sample hydrolyzes starch and the product is rapidly converted to glucose by  $\alpha$ -glucosidase and hydrogen peroxide by glucose oxidase; (2) hydrogen peroxide concentration is determined with a colorimetric reagent.

#### APPLICATIONS

Determination of  $\alpha$ -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  $\alpha$ -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

<b>Assay Buffer (pH 7.0):</b> 20 mL	<b>Substrate:</b> 120 $\mu$ L
<b>Detection Reagent:</b> 20 mL	<b>Enzyme A:</b> 120 $\mu$ L
<b>Glucose Standard:</b> 1 mL	<b>Enzyme B:</b> 120 $\mu$ L

**Storage conditions.** Kit is shipped on ice. Store all components at  $-20^{\circ}\text{C}$ . 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,  $\alpha$ -amylase is stable for one month. Ascorbic acid, heparin, EDTA, EGTA, citrate, SDS, Tris ( $> 8\text{mM}$ ) 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  $\mu\text{M}$  Glucose Standard by mixing 10  $\mu\text{L}$  of the provided (300 mg/dL) standard with 406  $\mu\text{L}$  Assay Buffer. Transfer 10  $\mu\text{L}$  Assay Buffer, 10  $\mu\text{L}$  400  $\mu\text{M}$  glucose, and 10  $\mu\text{L}$  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  $\mu\text{L}$  Assay Buffer, 0.5  $\mu\text{L}$  Substrate, 1  $\mu\text{L}$  Enzyme A, 1  $\mu\text{L}$  Enzyme B.

Transfer 40  $\mu\text{L}$  Working Reagent to each well. Incubate for 15 min at room temperature ( $25^{\circ}\text{C}$ ).

3. Add 150  $\mu\text{L}$  Detection Reagent to each well. Mix and incubate for 20 min at room temperature ( $25^{\circ}\text{C}$ ). Read OD<sub>585nm</sub> (540-610nm) on a plate reader.

#### CALCULATION

The Amylase activity is calculated as

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

OD<sub>SAMPLE</sub>, OD<sub>STD</sub> and OD<sub>BUFFER</sub> are optical density values of the sample, the 400  $\mu\text{M}$  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  $\mu\text{mole}$  of glucose per min under the assay conditions.

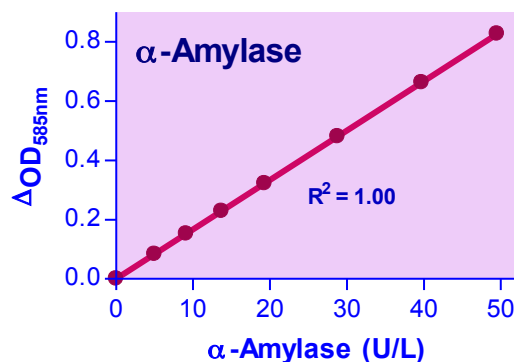
Note: if the calculated activity is higher than 50 U/L, dilute sample in Assay Buffer and repeat assay. Multiply the results by the dilution factor.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

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

#### GENERAL CONSIDERATIONS

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



Standard Curve in 96-well plate assay

#### LITERATURE

1. Gullbault, GG and Rietz, E.B. (1976). Enzymatic, Fluorometric Assay of  $\alpha$ -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.