QuantiChrom™ α-Amylase Assay Kit (DAMY2-100)

Quantitative Colorimetric Assay for α-Amylase Activity

DESCRIPTION

α-*AMYLASE* (EC 3.2.1.1) 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, as well as glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme. Increased α-amylase 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' QuantiChrom™ α-Amylase Assay Kit provides a convenient colorimetric method to measure α-amylase activity in biological samples. In this assay, α-amylase hydrolyzes a synthetic substrate to release 2-chloro-4-nitrophenol. The rate of formation of the 2-chloro-4-nitrophenol, measured at 405 nm, is directly proportional to the enzyme activity.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. Linear detection range of 0.2 - 100 U/L α-amylase in a 96-well plate assay.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of α-amylase activity in biological samples (saliva, serum, etc.).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Substrate: 0.5 mL**

Standard: 1 mL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. 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 the Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature (25°C) or 37°C.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The *Working Reagent* should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme can be prepared in Assay Buffer. The following protocol is optimized for porcine α-amylase from Calzyme (Cat #146A0100). Enzyme is not included with the kit. If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Sample Preparation:

Saliva may require a 2-fold or greater dilution in Assay Buffer.

Serum can be assayed directly.

Standard Preparation:

Mix 8 µL of 12.5 mM Nitrophenol standard with 492 µL dH_2O to make 200 µM standard.

No	200 µM STD + dH2O	Vol (µL)	Nitrophenol (µM)
1	100 µL + 0 µL	100	200
2	60 µL + 40 µL	100	120
3	30 µL + 70 µL	100	60
4	0 µL + 100 µL	100	0

Procedure using 96-well plate:

1. Transfer 100 µL of each standard (OD_{STD}) into wells of a clear flat bottom 96-well plate.
2. Transfer 10 µL of each sample into separate wells.
3. Prepare enough Working Reagent for each well by mixing 5 µL Substrate and 95 µL Assay Buffer. Add 90 µL Working Reagent to each sample well. Tap plate briefly to mix.
4. Incubate at room temperature or desired temperature for 20 minutes.
5. Read OD_{405nm}.

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate α-Amylase activity in the samples:

$$\alpha\text{-Amylase Activity} = \frac{OD_{SAMPLE} - OD_{BLANK}}{Time \bullet Slope} \times \frac{Reaction\ Vol\ (\mu L)}{Sample\ Vol\ (\mu L)} \times n$$

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \times 0.5 \times n \quad (U/L)$$

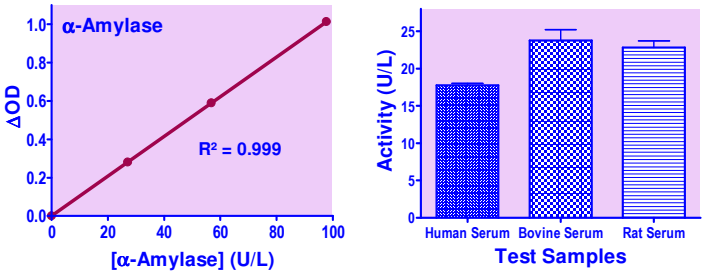
where OD_{SAMPLE} is the OD_{405nm} value for each sample and OD_{BLANK} is the OD_{405nm} value of the water (standard #4) or the sample blank if one was used. Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (20 min). Reaction Vol and Sample Vol are 100 µL and 10 µL, respectively. *n* is the dilution factor.

Unit definition: 1 Unit (U) of α-amylase will catalyze the conversion of 1 µmole of the Substrate per min at assay temperature and pH 7.0.

Note: If sample α-amylase activity exceeds 100 U/L, dilute samples in assay buffer and repeat the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader.



Left: Titration Curve of α-amylase in a 96-well plate. Right: Base activity values in serum samples. α-amylase activity was determined to be 17.75 U/L, 23.78 U/L, and 22.82 U/L in human, bovine, and rat serum samples, respectively. These values are examples and are not intended as expected values.

LITERATURE

1. Janeček Š. et al. (2014). α-Amylase: an enzyme specificity found in various families of glycoside hydrolases. Cell Mol Life Sci. 71(7):1149-70.

2. Petrakova, L. et al. (2015). Psychosocial Stress Increases Salivary Alpha-Amylase Activity Independently from Plasma Noradrenaline Levels. PLoS ONE 10(8).

3. Sun, L. et al. (2018). Cloning, expression, and characterization of a porcine pancreatic α-amylase in Pichia pastoris. Animal Nutrition. 4(2), 234-240.