Alpha-Amylase Assay Kit

Product Datasheet | SKU BA0267

Description

Alpha-amylase (EC 3.2.1.1) 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 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, alpha-amylase is a major digestive enzyme, and increased levels in humans are associated with salivary trauma, mumps due to inflammation of the salivary glands, pancreatitis and renal failure. This kit provides a convenient colorimetric method to measure alpha-amylase activity in biological samples, in which the enzyme hydrolyses a synthetic substrate to release 2-chloro-4-nitrophenol; the rate of formation, measured at 405 nm, is directly proportional to enzyme activity.

Specifications

Detection Method	Colorimetric (OD 405 nm)
Detection Range	0.2 - 100 U/L
Sample Type	Biological samples (saliva, serum, etc.)
Species Reactivity	All
Assay Time	20 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
Substrate	0.5 mL	-20°C
Standard	1 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0267	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature and briefly centrifuge tubes before opening; the Working Reagent should be prepared fresh for each assay run.
2. Prepare samples: saliva may require a 2-fold or greater dilution in Assay Buffer, while serum can be assayed directly.
3. Prepare a 200 µM nitrophenol standard by mixing 8 µL of 12.5 mM standard with 492 µL distilled water, then dilute to give standards of 200, 120, 60 and 0 µM in a final volume of 100 µL.
4. Transfer 100 µL of each standard into wells of a clear flat-bottom 96-well plate and transfer 10 µL of each sample into separate wells.
5. 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 and tap the plate briefly to mix.

6. Incubate at room temperature (25°C) or 37°C for 20 minutes and read OD405nm.

Standard Preparation

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

Calculation

Subtract blank OD (water, #4) from the standard OD values and plot delta-OD against standard concentrations to determine the Slope. Alpha-Amylase Activity = (ODSAMPLE - ODBLANK) / Slope x 0.5 x n (U/L), where ODSAMPLE is the OD405nm value for each sample, ODBLANK is the OD405nm of the water (standard #4) or sample blank, and n is the dilution factor. One unit (U) of alpha-amylase catalyses the conversion of 1 µmole of substrate per minute at assay temperature and pH 7.0.

Notes

- This assay is based on a kinetic reaction; addition of the Working Reagent to samples should be quick and mixing brief but thorough, with a multi-channel pipettor recommended.
- Enzyme can be prepared in Assay Buffer; the protocol is optimised for porcine alpha-amylase and enzyme is not included with the kit, so the optimal amount per well should be determined experimentally for other enzymes.
- If sample alpha-amylase activity exceeds 100 U/L, dilute samples in assay buffer and repeat the assay.
- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.