

α-Amylase Activity Colorimetric Assay (Catalog: TBS2207, 100 Assays, Store at -20 °C)

DESCRIPTION

α-Amylase is an essential enzyme that catalyzes the breakdown of starch and glycogen into simpler sugars, playing a vital role in the digestive system. The assay utilizes a chromogenic substrate; α-amylase hydrolyzes this substrate to release 2-chloro-4-nitrophenol (CNP), which produces a yellow color that is measurable at 405 nm. The rate of increase in absorbance is directly proportional to the α-amylase activity in the sample. When amylase levels in the blood are elevated or reduced, it can indicate pancreatic or salivary gland disorders, gallstones, cancers, or other metabolic diseases, making it a key marker in diagnosing or monitoring these conditions.

Tribioscience Amylase Activity Colorimetric Assay is based on an amylase reaction that converts a chromophore to a colored formazan, which can be measured at OD 405 nm. The signal generated is proportional to the content of CNP as a product of the amylase enzyme. The kit provides the easiest and most accurate approach to measure amylase activity from a variety of samples.

Synonyms: Amylolytic enzyme, Carbohydrase, 1,4-α-D-Glucan-glycanohydrolase.

APPLICATIONS

Specifically detect amylases from cell and biological tissues.

KIT CONTENTS FOR 100 TESTS:

Name	Part Size
Assay Buffer	10 mL
Substrate (5 x)	1 mL
Standard stock (100 mM)	10 μL
Positive enzyme control	110 μL

Storage conditions: Store the Reagent at -20 °C for a year.

PROCEDURES

1. Sample preparation

- 1) Wash the cells with 400 μL cold PBS.
- 2) Pellet 2 X 10⁵ cells at 2000 rpm for 5 min.
- 3) Aspirate the wash solution from the tube.
- 4) Homogenization in 150 μL assay buffer for 30 sec.
(For tissues, weigh ~20 mg tissue & wash with cold PBS. Homogenize in Assay Buffer in a micro-centrifuge tube).
- 5) Centrifuge at 14000 rpm for 5 min.
- 6) Transfer the extracted supernatant into 2 labeled tubs.
- 7) To transfer 50 μL of extracted samples into labeled 96-well plate.

2. Prepare standards as below Table 1.

Table 1: Amylase Standard CPN Preparation

Std	Standard (μL)	Assay buffer (μL)	μM	nmol/well
1	3 (100 mM stock)	297	1000	50
2	150 (Std1)	150	500	25
3	150 (Std2)	150	250	12.5
4	150 (Std3)	150	125	6.25
5	150 (Std4)	150	62.5	3.125
6	150 (Std5)	150	31.2	1.56
7	150 (Std6)	150	15.6	0.78
8	0	150	0	0

3. Prepare substrate working solution for 50μl/well:

10 μL of substrate (5x) + 40 μL of assay buffer

4. Set up reaction

Add 50 μL of Standard or test sample preparation, and positive control or blank (assay buffer) into the indicated well. *(Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).*

5. Add 50 μL of substrate working solution to each well containing the test samples, positive control and blank. Then add 50 μL of assay buffer to the standards.

6. Initiate Reaction:

Incubate at 37°C for about 60-90 minutes, with gentle shaking and protecting from light.

7. Measure OD value at 405 nm:

The amylase standard and samples can be read at the end point.

8. Calculation using standard curve:

The standard values subtract the blank value (0 μM Standard) and plot the ΔOD against standard concentrations. Determine the slope and calculate the amylase concentration of the sample using the equation obtained from the linear regression of the standard curve.

$$\text{Amylase Activity (U/L)} = \text{DF} * (\text{ODSAMPLE} - \text{OD BLANK}) / (\text{t} * \text{Slope})$$

Where: R sample and R blank are optical density readings of the sample and blank, respectively. DF is the sample dilution factor. Slope is the linear regression fit of the standard points and t is the reaction time (60 or 90 min).

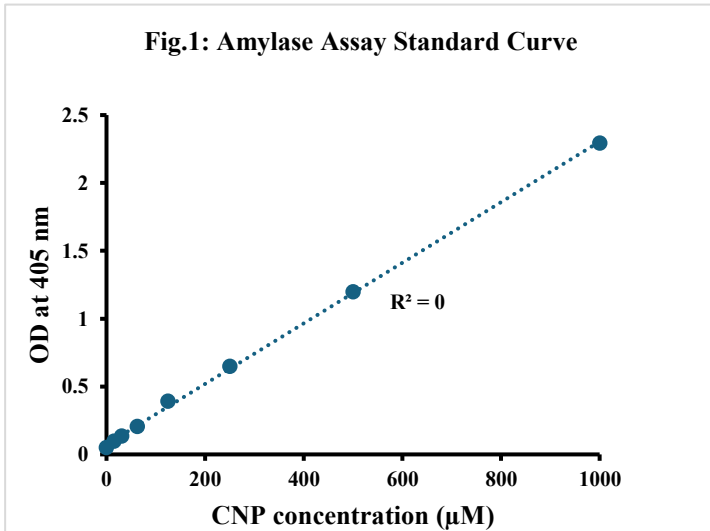
Note: If unknown sample results are over standard curve range, dilute samples with assay buffer, and repeat the assay.

Unit definition: 1 Unit (U) definition that amylase converse of 1 μmole of substrate to CNP per min at 37°C.

α-Amylase Activity Colorimetric Assay
(Catalog: TBS2207, 100 Assays, Store at -20 °C)

TYPICAL STANDARD CURVE

The Typical Standard curve showed as an example in Fig.1. It can be used only for reference.



RELATIVE PRODUCTS

- Resazurin Cell Viability Kit (TBS2001)
- LDH Cytotoxicity Assay (TBS2002)
- Caspase-3 Colorimetric Assay kit (TBS2030)
- CD38 Cyclase Activity Assay (TBS2100)
- LDH Activity Assay (TBS2012)
- CCK-8 Cell Viability Assay (TBS2022)
- GOT Activity Assay (TBS2013)
- Thiol Fluorometric Assay (TBS2026)
- GSH Assay (TBS2028)
- Homocysteine Fluorometric Assay (TBS2091)
- AHCY Inhibitor Screening Assay (TBS2097)
- G6PDH Activity Colorimetric Assay (TBS2102)
- ATP Colorimetric/Fluorometric Assay (TBS2010)
- ADP Colorimetric / Fluorometric Assay (TBS2020)
- NNMT Activity Assay (TBS2098)
- NADP Assay (TBS2058)
- NAD/NADH Assay (TBS2029)

For research use only.

The positive control graph is shown in Fig.2 as below.

