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Product Information

Amylase Activity Assay Kit

Catalog Number **MAK009**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Amylase enzymes are glycoside hydrolases, which cleave glucan linkages in polysaccharides such as starch or glycogen to release polysaccharide molecules. α-Amylase is found in animals, plants, and many fungi. In mammals, α-amylase is the predominant form of amylase and is one of the major enzymes of dietary digestion. Both the activity and the stability of α-amylase is dependent on calcium ions. In humans, α-amylase levels are highest in pancreatic and salivary secretions. Increased blood and urine levels of amylase occur due to pancreatic disorders and salivary trauma. Increased salivary amylase activity is also a marker for increased sympathetic nervous system activity.

The Amylase Activity Assay kit quantifies amylase activity in a variety of biological samples. The assay is quick, convenient, and sensitive. In this kit, amylase activity is determined using a coupled enzymatic assay, which results in a colorimetric (405 nm) product, proportional to the amount of substrate, ethylidene-pNP-G7, cleaved by the amylase. One unit is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 μmole of *p*-nitrophenol per minute at 25 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

Amylase Assay Buffer Catalog Number MAK009A	55 mL
Amylase Substrate Mix Catalog Number MAK009B	5 mL
Amylase Positive Control Catalog Number MAK009C	1 vl
Nitrophenol Standard Catalog Number MAK009D	150 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Amylase Assay Buffer – Allow buffer to come to room temperature before use.

Amylase Positive Control – Reconstitute with 50 μL of Amylase Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples, controls, and standards should be run in duplicate.

<u>Nitrophenol Standards for Colorimetric Detection</u>
Add 0, 2, 4, 6, 8, 10 μL of the 2 mM Nitrophenol Standard into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 nmole/well standards. Add water to each well to bring the volume to 50 μL.

Sample Preparation
Tissue (100 mg) or cells (4×10^6) can be homogenized in 0.5 mL of the Amylase Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum and urine samples can be directly added to wells.

Add 1–50 µL of sample into wells of a 96 well plate. Bring samples to a final volume of 50 µL with Amylase Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For the positive control (optional), add 5 µL of the Amylase Positive Control solution to wells and adjust to 50 µL with the Amylase Assay Buffer

Assay Reaction
1. Prepare the Master Reaction Mix according to Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Amylase Assay Buffer	50 µL
Amylase Substrate Mix	50 µL

2. Add 100 µL of the Master Reaction Mix to each of the sample, standard, and positive control wells. Mix well using a horizontal shaker or by pipetting.

3. After 2–3 minutes ($T_{initial}$), measure the absorbance at 405 nm $(A_{405})_{initial}$.
   Note: It is essential that $(A_{405})_{initial}$ is in the linear range of the standard curve.

4. Incubate the plate at 25 °C, measuring the absorbance (A_{405}) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (20 nmole/well). At this time, the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final absorbance measurement $[(A_{405})_{final}]$ for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results
Calculations
Correct for the background by subtracting the final measurement $(A_{405})_{final}$ obtained for the 0 (blank) nitrophenol standard from the $(A_{405})_{final}$ measurement of the standards and samples.
Note: A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{405} = (A_{405})_{final} - (A_{405})_{initial}$$

Compare the ΔA_{405} of each sample to the standard curve to determine the amount of nitrophenol (B) generated by the amylase between $T_{initial}$ to T_{final}.

The amylase activity of a sample may be determined by the following equation:

$$\text{Amylase Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of nitrophenol generated between $T_{initial}$ and T_{final}
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

Amylase activity reported as nmole/min/mL (milliunits). One unit of amylase is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 µmole of *p*-nitrophenol per minute at 25 °C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until used
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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