Product Information

Amylase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2225-100 100 assays	Cap Color	Part Number
Amylase Assay Buffer	55 ml	NM	K2225-C-1
Amylase Substrate Mix	5 ml	NM	K2225-C-2
Amylase Positive Control (lyophilized)	1 vial	Red	K2225-C-3
Nitrophenol Standard (2 mM)	150 µl	Yellow	K2225-C-4

II. Introduction:

Amylase is an enzyme that break starch down to sugar. Amylase is present in the saliva of humans. α-Amylase is the major form found in fungi, seeds, humans and other mammals. α-Amylase is a calcium metalloenzyme which completely unable to function in the absence of calcium. In humans, both the pancreatic and salivary amylases are major digestive enzymes. Increased amylase levels in humans are associated with salivary trauma, mumps because of renal failure, pancreatitis and inflammation of the salivary glands.

The Amylase Activity Colorimetric Assay Kit provides a sensitive, simple, fast and convenient way for detection of α-amylase activity in various samples based on colorimetric method. The assay utilizes ethylidene-pNP-G7 as the substrate. In the assay, the substrate is specifically cleaved by α-amylase to produce the smaller fragments, which can be acted upon by α-glucosidase that causes the ultimate release of the chromophore (at 405 nm). The kit can detect as low as 0.2 mU α-amylase content.

III. Reagent Reconstitution and General Consideration:

Store kit at -20℃ Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. Keep samples and amylase positive control on ice during the assay. Amylase Positive Control: Dissolve into 50 µl Assay Buffer, and store at -20℃.

IV. Amylase Activity Assay:

1. Sample and Positive Control Preparations:

Serum and urine samples can be tested directly. Add 0.5 - 50 µl samples or 5 µl Amylase Positive Control into each well, and adjust volume to 50 µl with dH_2O. Tissue (100 mg) or cells (4×10^6) can be extracted with 0.5 ml Assay Buffer and centrifuged at 16,000 x g for 10 min. The clear extract can be assayed directly. For unknown samples, we suggest using different doses to ensure the readings are within the linear range.

2. Nitrophenol Standard Curve:

Add 0, 2, 4, 6, 8, 10 µl of 2 mM nitrophenol Standard mix into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well nitrophenol standard. Bring the total volume to 50 µl with dH_2O.

3. Reaction Mix: Prepare enough reaction mix for samples, standard and positive control. For each reaction:

Assay Buffer 50 µl

Substrate Mix 50 µl

4. Add 100 µl of the reaction mix into each reaction and mix. Measure immediately (T0) at OD 405 nm to get ODT0. Incubate the reaction at 25℃ for various times (T1) and measure OD 405 nm to get ODT1 (Sample incubation time can vary depending on α-amylase activity in samples. We

recommend observing the reaction kinetics then choosing the linear range for T0-T1. The Standard Curve will not change as incubation time increases).

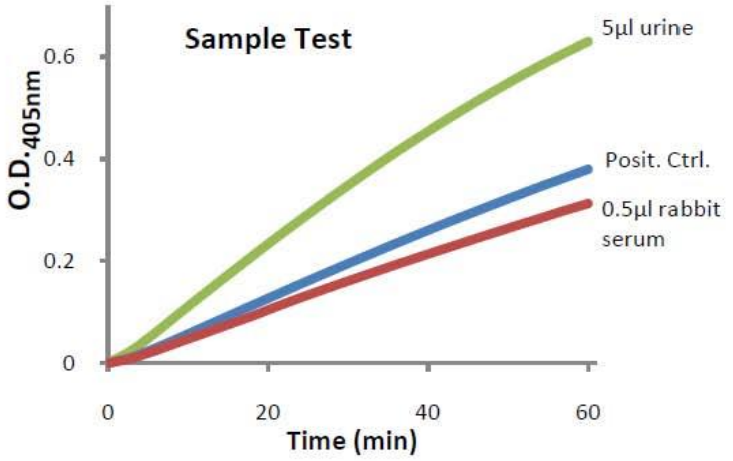
5. Calculation: Plot the Nitrophenol standard curve. Apply the ΔOD (ΔOD = ODT1 -ODT0) to the Nitrophenol standard curve to get B nmol of Nitrophenol generated by amylase between T0 and T1. Amylase Activity = B /(Tx V) x Sample Dilution Factor = nmol/min/ml = mU/mL

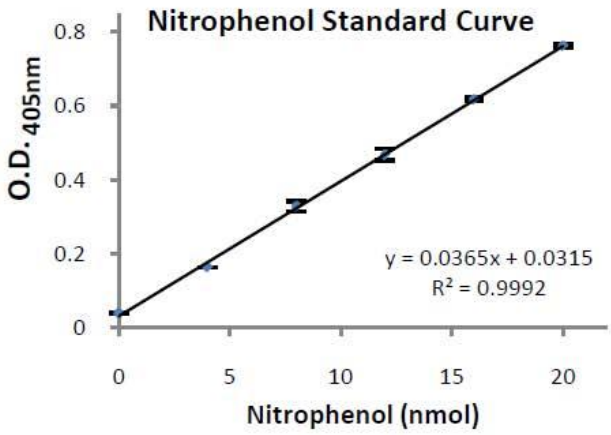
Where: B is the Nitrophenol amount from Standard Curve (in nmol).

T is the time between T0 and T1 (in min).

V is the pretreated sample volume added to the reaction well (in ml).

Unit Definition: One unit of amylase is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 µmol of nitrophenol per min at pH 7.20 at 25℃





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in	• Improperly thawed components • Use of expired kit or improperly stored reagents	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components

Samples and Standards	• Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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