

Amylase Activity Assay Kit

(Catalog# K711-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Amylases are enzymes that break starch down to sugar molecules. α-amylase is the major form of amylase found in humans and other mammals as well as an enzyme present in seeds, or in fungi (baker's yeast for instance). α-amylase is a calcium metalloenzyme, completely unable to function in the absence of calcium. In human physiology, both the salivary and pancreatic amylases are major digestive enzymes. Increased enzyme levels in humans are associated with salivary trauma; mumps due to inflammation of the salivary glands, pancreatitis and renal failure. A simple, direct and automation-ready procedure for measuring α-amylase activity is, therefore, very desirable. BioVision's α-amylase Assay uses ethylidene-pNP-G7 as the substrate. Once the substrate has been specifically cleaved by α-amylase, the smaller fragments produced can be acted upon by α-glucosidase, which causes the ultimate release of the chromophore that can then be measured at 405 nm. The assay can detect α-amylase content as low as 0.2 mU.

II. Kit Contents:

Component	K711-100	Cap Color	Part Number
Amylase Assay Buffer	25 ml	WM	K711-100-1
Amylase Substrate Mix	5 ml	NM	K711-100-2
Amylase Positive Control (lyophilized)	1 vial	Red	K711-100-3
Nitrophenol Standard (2 mM)	150 µl	Yellow	K711-100-4

III. Reagent Reconstitution and General Consideration:

Store kit at -20°C. 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°C.

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 dH₂O. Tissue (100 mg) or cells (4 x 10⁶) 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 dH₂O.

3. Reaction Mix:

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

- 50 µl Assay Buffer
- 50 µl Substrate Mix

4. Add 100 µl of the reaction mix into each reaction and mix. Measure immediately (T₀) at OD 405 nm to get OD_{T0}. Incubate the reaction at 25°C for various times (T₁) and measure OD 405 nm to get OD_{T1} (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:

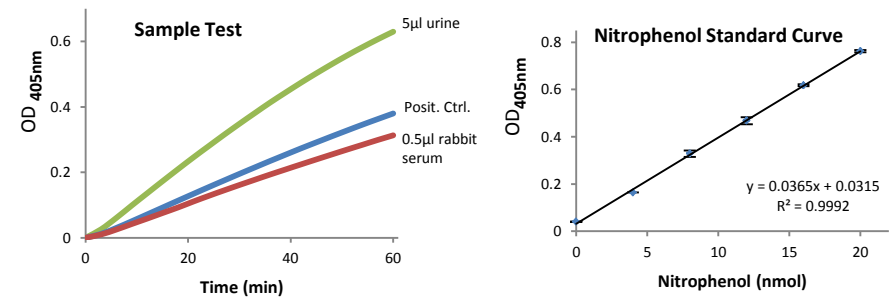
Plot the Nitrophenol standard curve. Apply the ΔOD (ΔOD = OD_{T1} - OD_{T0}) to the Nitrophenol standard curve to get B nmol of Nitrophenol generated by amylase between T₀ and T₁.

$$\text{Amylase Activity} = \frac{B}{T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

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 °C.



RELATED PRODUCTS:

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Colorimetric Glutathione Detection Kit
Glutathione Kit (GSH, GSSG and Total)
GST Colorimetric Assay Kit
Acid Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
NAD/NADH Quantification Kit
Pyruvate Assay Kit
Glutamate Assay Kit
Ethanol Assay Kit
Amino Acid Assay Kit
Ascorbic Acid Assay Kit
Ethanol Assay Kit
Cholesterol Assay Kit
Triglyceride Assay Kit
SOD Assay Kit
Acid Phosphatase Assay Kit
cAMP & cGMP Assay Kits
Beta-Secretase Assay Kit

Lactate Assay Kits
ApoGSH Glutathione Detection Kit
GST Fluorometric Assay Kit
Triglyceride Assay Kit
ADP/ATP Ratio Assay Kit
Phosphate Colorimetric Assay Kit
NADP/NADPH Quantitation Kit
Ammonia Assay Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit
Sucrose Assay Kit
Galactose Assay Kit
Maltose Assay Kit
LDH & LDL/VLDL Assay Kit
Choline Assay Kit
Phosphate Assay Kit
Alkaline Phosphatase Assay Kit
Protein Kinase Assay Kits
Urea Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • 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 (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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 • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • 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.		