α-Amylase Activity Colorimetric 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	55 ml	NM	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 (RT) 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 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 duplicates to generate 0, 4, 8, 12, 16, 20 nmol/well Nitrophenol Sstandard. Bring the total volume to 50 µl with dH_2O.

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

50 µl Assay Buffer
50 µl Substrate Mix

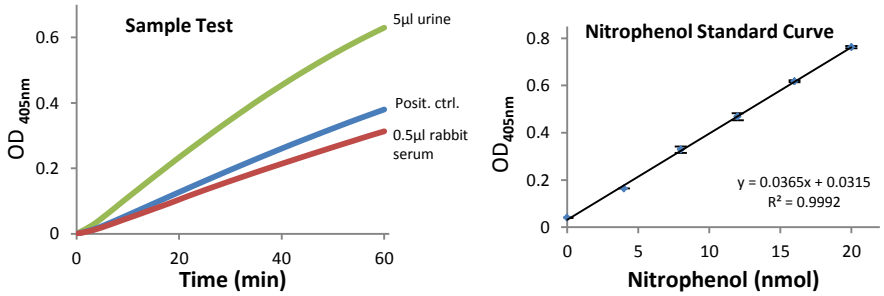
4. Add 100 µl of the reaction mix into each reaction and mix. Measure immediately (T_0) at OD 405 nm to get OD_{T0}. Incubate the reaction at 25 °C for various times (T_1) and measure OD 405 nm to get OD_{T1} (Sample incubation time can vary depending on α-amylase activity in samples. We recommend observing the reaction kinetics then choosing the linear range for T_0-T_1. The Standard Curve will not change as incubation time increases).

5. Calculation: Plot the Nitrophenol Standard Curve. Apply the ΔOD ($\Delta OD = OD_{T1} - OD_{T0}$) to the Nitrophenol Standard Curve to get B nmol of Nitrophenol generated by amylase between T_0 and T_1.

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

Where: **B** is the Nitrophenol amount from the Standard Curve (in nmol).
T is the time between T_0 and T_1 (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 7.2 at 25 °C.



RELATED PRODUCTS:

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Colorimetric Glutathione Detection Kit
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GST Colorimetric Assay Kit
Acid Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
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Pyruvate Assay Kit
Glutamate Assay Kit
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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	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		