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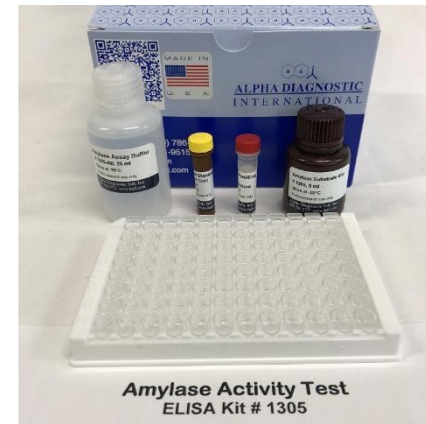
Instruction Manual No. M-1305

Amylase Activity Test

Cat. # 1305

Colorimetric Microplate Assay Kit

For Quantitative Amylase Activity in Serum, Plasma, Urine, or Tissues Samples



For In Vitro Research Use Only

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Amylase KIT # 1305 Kit Contents: (reagents for 96 tests)

Kit Contents	#
96-well microplate (8 wells x 12 strips), #1306P	1 Pate
Nitrophenol Standard (2 mM) **150 µl** Yellow cap, #1307	1 vial
Amylase **Positive Control (lyophilized)**, #1308	1 vial
Amylase **Substrate Mix** 5 ml, # 1309	1 bottle
Amylase **Assay Buffer** 25 ml, 1305-AB	1 bottle
Instruction Manual, M-1305	1
Store kit at -20°C	

Intended Use

Amylase enzyme activity kit is for Quantitative Amylase Activity in Serum, Plasma, Urine, or Tissues Samples. The kit can be used for any species. For in vitro research use only.

1.Introduction

Amylases are enzymes that break starch down to sugar molecules. a-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). a-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 a-amylase activity is therefore, very desirable. ADI's- amylase Assay uses ethylidene-pNP-G7 as the substrate. Once the substrate has been specifically cleaved by a-amylase, the smaller fragments produced can be acted upon by a-glucosidase, which causes the ultimate release of the chromophore that can then be measured at 405 nm. The assay can detect a- amylase content as low as 0.2 mU.

2. Reagent Reconstitution and General Consideration:

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: 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.

3. 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 Amylase Assay buffer.

Tissue (100 mg) or cells (4 x 10^6) can be extracted with 0.5 ml of Amylase Assay Buffer and centrifuged at 13,000 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.

Amylase assay procedure

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

50 µl Assay Buffer + 50 µl Substrate Mix or 100 ul total. For each strip of 8 wells prepare 1 ml or 10 ml for the entire plate (5 ml of assay buffer and 5 ml of Substrate Mix)

Prepare Amylase Standards:

1. Add 0, 2, 4, 6, 8, 10 µl of 2 mM nitrophenol standard 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 water.
2. Add 100 µl of the reaction mix into each of the sample, standard and positive control wells. Mix well using a horizontal shaker or by pipetting. After 2-3 minutes, Measure absorbance at OD 405 nm to get OD (T0). Incubate the plate at 25°C, measuring the absorbance every 5 minutes. Protect the plate from light during incubation.
3. Continue taking measurements until the value of most active sample is greater than the value of highest standard (20 nmole/well). At this time, the most active sample is near or exceeds the end of linear range of the Standard curve.
4. The final absorbance measurement [T1] 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 Tfinal (T1). Note: It is essential the final measurement falls within the linear range of the standard curve.

Calculation:

Plot the Nitrophenol standard curve. Apply the ∆OD (∆OD = ODT1-ODT0) to the Nitrophenol standard curve to get B nmol of Nitrophenol generated by amylase between 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 generated between T1 and T0 (in nmol).
T (Reaction Time) is T1 – T0 (minutes)
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.