ab102523 Amylase Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Amylase activity in various samples. This product is for research use only and is not intended for diagnostic use.

Materials Supplied and Storage

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components.
Aliquot components in working volumes before storing at the recommended temperature.
Reconstituted components are stable for 2 months.

Item	Amount	Storage Condition (Before Prep.)	Storage Condition (After Prep.)
Amylase Assay Buffer	55 mL	-20°C	-20°C
Amylase Substrate Mix	5 mL	-20°C	-20°C
Amylase Positive Control	1 vial	-20°C	-20°C
Nitrophenol Standard	400 µL	-20°C	-20°C

Materials Required, Not Supplied

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 405 nm
- 96 well plate: clear plate for colorimetric assay
- Orbital shaker
- Vortex
- Dounce homogenizer or pestle (if using tissue)

1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

1.1 Amylase Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

1.2 Amylase Substrate Mix: Ready to use as supplied. Keep on ice during the assay. Aliquot substrate so that you have enough to perform the desired number of tests. Store at -20°C.

1.3 Amylase Positive Control: Dissolve Amylase Positive Control into 50 µL Assay Buffer. Aliquot positive control so that you have enough to perform the desired number of tests. Keep positive control on ice during the assay. Store at -20°C.

1.4 Nitrophenol Standard: Ready to use as supplied. Aliquot standard so that you have enough to perform the desired number of tests. Store at -20°C.

2. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

2.1 Using 2mM Nitrophenol standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Nitrophenol] in well
1	0	150	50	0 nmol/well
2	6	144	50	4 nmol/well
3	12	138	50	8 nmol/well
4	18	132	50	12 nmol/well
5	24	126	50	16 nmol/well
6	30	120	50	20 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

3. Sample Preparation

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

3.1 Cell (adherent or suspension) samples:

3.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 4 x 10^6 cells).
3.1.2 Wash cells with cold PBS.
3.1.3 Resuspend cells in 500 µL Amylase Assay Buffer.
3.1.4 Homogenize cells quickly by pipetting up and down a few times.
3.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
3.1.6 Collect supernatant and transfer to a clean tube.
3.1.7 Keep on ice.

3.2 Tissue samples:

3.2.1 Harvest the amount of cells necessary for each assay (initial recommendation = 100 mg tissue).
3.2.2 Wash tissue in cold PBS.
3.2.3 Resuspend tissue in 500 µL Amylase Assay Buffer.
3.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
3.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed, using a cold microcentrifuge to remove any insoluble material.
3.2.6 Collect supernatant and transfer to a clean tube.
3.2.7 Keep on ice.

3.3 Serum and Urine:

3.3.1 Serum and urine samples can be tested directly by adding sample to the microplate wells.

3.3.2 However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

4. Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
It is recommended to assay all standards, controls and samples in duplicate.

4.1 Set up Reaction wells:

Standard wells = 50 µL Standard dilutions.
Positive control wells – Add 5 µL of Amylase Positive Control and adjust to 50 µL/well with ddH_2O.
Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with ddH_2O).

4.2 Reaction Mix:

Prepare 100 µL of Reaction Mix for each reaction:

Component	Reaction Mix Samples (µL)
Amylase Assay Buffer	50
Amylase Substrate Mix	50

Mix enough reagents for the number of assays (samples, standards, positive control and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

4.3 Add 100 µL of Reaction Mix into each well. Mix thoroughly.

4.4 Measure absorbance immediately at OD=405 nm in a kinetic mode, every 2 – 3 minutes, for 30 – 60 min at 25°C protected from light.

NOTE: *Sample incubation time can vary depending on a-amylase activity in samples. We recommend observing the reaction kinetics then choosing two time points (T1 and T2) in the linear range to calculate the amylase activity. The Standard Curve will not change as incubation time increases.*

5. Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

5.1 Average the duplicate reading for each standard and sample.

5.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

5.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Nitrophenol.

5.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

5.5 Activity of amylase is calculated as:

$$\Delta A_{405nm} = A_2 - A_1$$

Where:
A1 is the sample reading at time T1
A2 is the sample reading at time T2

5.6 Use the ΔA405nm to obtain B nmol of Nitrophenol generated by amylase during the reaction time (ΔT = T2 – T1).

5.7 Activity of Amylase in the test samples is calculated as:

$$\textbf{Amylase Activity} = \left(\frac{B}{\Delta T x\, V}\right) * D$$

Amylase = nmol/min/mL = mU/mL
Where:
B = Nitrophenol amount from the standard curve (in nmol).
ΔT = reaction time (T2 – T1) (min).
V = Pretreated sample volume added to the reaction well (in mL).
D = sample dilution factor.

Unit Definition:
1 Unit Amylase = amount of amylase that cleaves ethylidene-*p*NP-G7 to generate 1.0 µmol of nitrophenol per min at pH 7.20 at 25 °C.

FAQs

Will this kit work for beta or gamma amylases as well?
This kit is specific for alpha-amylase activity.

Is there an upper limit for this kit regarding alpha amylase concentration?
To be able to calculate Amylase activity in samples, it is essential to get values within the linear range of the standard curve. The highest datapoint for the standard is 20 nmol of Nitrophenol per well. As mentioned on our datasheet, 1 unit of amylase generates 1.0 µmol of nitrophenol per min at pH 7.20 at 25 °C. Hence the maximum amount of enzyme activity within the linear range of the standard curve would be 20 mU per well. The sample amount needed to get values within the linear range of the std. curve is sample dependent and has to be optimized in a pilot assay by the user.

For all technical or commercial enquiries please go to:
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