

**ab102523**

# **Amylase Assay kit (Colorimetric)**

Instructions for Use

For the rapid, sensitive and accurate measurement of Amylase activity in various samples.

[View kit datasheet: www.abcam.com/ab102523](http://www.abcam.com/ab102523)

(use [www.abcam.cn/ab102523](http://www.abcam.cn/ab102523) for China, or [www.abcam.co.jp/ab102523](http://www.abcam.co.jp/ab102523) for Japan)

This product is for research use only and is not intended for diagnostic use.



# Table of Contents

---

## INTRODUCTION

- 1. BACKGROUND 2
- 2. ASSAY SUMMARY 3

## GENERAL INFORMATION

- 3. PRECAUTIONS 4
- 4. STORAGE AND STABILITY 4
- 5. MATERIALS SUPPLIED 5
- 6. MATERIALS REQUIRED, NOT SUPPLIED 5
- 7. LIMITATIONS 6
- 8. TECHNICAL HINTS 7

## ASSAY PREPARATION

- 9. REAGENT PREPARATION 8
- 10. STANDARD PREPARATION 9
- 11. SAMPLE PREPARATION 10

## ASSAY PROCEDURE and DETECTION

- 12. ASSAY PROCEDURE and DETECTION 12

## DATA ANALYSIS

- 13. CALCULATIONS 13
- 14. TYPICAL DATA 15

## RESOURCES

- 15. QUICK ASSAY PROCEDURE 17
- 16. TROUBLESHOOTING 18
- 17. FAQ 20
- 18. INTERFERENCES 21
- 19. NOTES 22

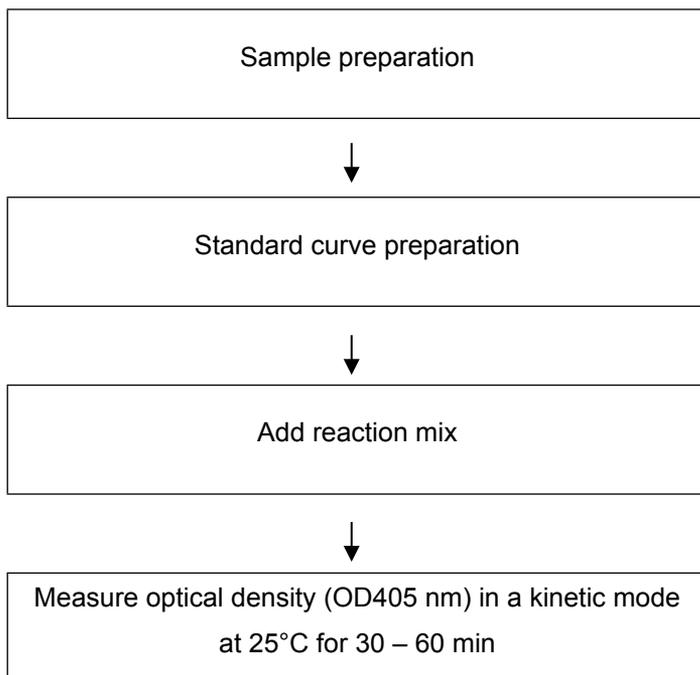
## 1. BACKGROUND

Amylase Assay kit (Colorimetric) (ab102523) detects activity of  $\alpha$ -amylase through a two-step reaction.  $\alpha$ -Amylase will cleave the substrate ethylidene-pNP-G7 to produce smaller fragments that are eventually modified by  $\alpha$ -glucosidase, causing the release of a chromophore that can then be measured at OD = 405 nm. The assay can detect  $\alpha$ -amylase content as low as 0.2 mU.

Amylases are enzymes that break starch down to sugar molecules.  $\alpha$ -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).  $\alpha$ -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  $\alpha$ -amylase activity is, therefore, very desirable.

## 2. ASSAY SUMMARY



*\*For kinetic mode detection, incubation time given in this summary is for guidance only.*

### **3. PRECAUTIONS**

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

### **4. STORAGE AND STABILITY**

**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 in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

**5. MATERIALS SUPPLIED**

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Amylase Assay Buffer	55 mL	-20°C	-20°C
Amylase Substrate Mix	5 mL	-20°C	-20°C
Amylase Positive Control (Lyophilized)	1 vial	-20°C	-20°C
Nitrophenol Standard (2mM)	150 µL	-20°C	-20°C

**6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH<sub>2</sub>O)
- 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)

### 7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

### 8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

## 9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **Amylase Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

### 9.2 **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.

### 9.3 **Amylase Positive Control:**

Dissolve Amylase Positive Control into 50  $\mu$ L 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.

### 9.4 **Nitrophenol Standard (2 mM):**

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

## 10. STANDARD PREPARATION

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

10.1 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).

## 11. SAMPLE PREPARATION

### **General Sample information:**

- 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  $-80^{\circ}\text{C}$ . 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.

### **11.1 Cell (adherent or suspension) samples:**

- 11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation =  $4 \times 10^6$  cells).
- 11.1.2 Wash cells with cold PBS.
- 11.1.3 Resuspend cells in 500  $\mu\text{L}$  Assay Buffer.
- 11.1.4 Homogenize cells quickly by pipetting up and down a few times.
- 11.1.5 Centrifuge sample for 2 – 5 minutes at  $4^{\circ}\text{C}$  at top speed using a cold microcentrifuge to remove any insoluble material.
- 11.1.6 Collect supernatant and transfer to a clean tube.
- 11.1.7 Keep on ice.

### **11.2 Tissue samples:**

- 11.2.1 Harvest the amount of cells necessary for each assay (initial recommendation = 100 mg tissue).
- 11.2.2 Wash tissue in cold PBS.
- 11.2.3 Resuspend tissue in 500  $\mu\text{L}$  Assay Buffer.

- 11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
  - 11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed, using a cold microcentrifuge to remove any insoluble material.
  - 11.2.6 Collect supernatant and transfer to a clean tube.
  - 11.2.7 Keep on ice.
- 11.3 **Serum and Urine:**

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

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.*

## 12. ASSAY PROCEDURE and DETECTION

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

### 12.1 Set up Reaction wells:

- Standard wells = 50  $\mu$ L Standard dilutions.
- Positive control wells – Add 5  $\mu$ L of Amylase Positive Control and adjust to 50  $\mu$ L/well with ddH<sub>2</sub>O.
- Sample wells = 2 – 50  $\mu$ L samples (adjust volume to 50  $\mu$ L/well with ddH<sub>2</sub>O).

### 12.2 Reaction Mix:

Prepare 100  $\mu$ L of Reaction Mix for each reaction:

Component	Reaction Mix Samples ( $\mu$ L)
Assay Buffer	50
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:

$X \mu\text{L component} \times (\text{Number samples} + \text{standards} + 1)$

- 12.3 Add 100  $\mu$ L of Reaction Mix into each well. Mix thoroughly.
- 12.4 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  $\alpha$ -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.*

## 13. 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).

13.1 Average the duplicate reading for each standard and sample.

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

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

13.4 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).

13.5 Activity of amylase is calculated as:

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

Where:

A1 is the sample reading at time T1

A2 is the sample reading at time T2

13.6 Use the  $\Delta A_{405\text{nm}}$  to obtain B nmol of Nitrophenol generated by amylase during the reaction time ( $\Delta T = T2 - T1$ ).

13.7 Activity of LDH in the test samples is calculated as:

$$\text{Amylase Activity} = \left( \frac{B}{\Delta T \times V} \right) * D$$

Amylase = nmol/min/mL = mU/mL

Where:

B = Nitrophenol amount from the standard curve (in nmol).

$\Delta T$  = reaction time ( $T_2 - T_1$ ) (min).

V = Pretreated sample volume added to the reaction well (in mL).

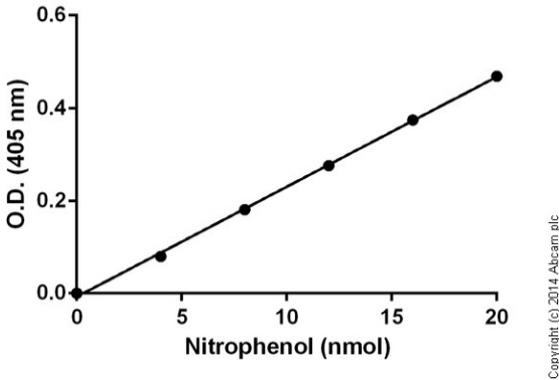
D = sample dilution factor.

### **Unit Definition:**

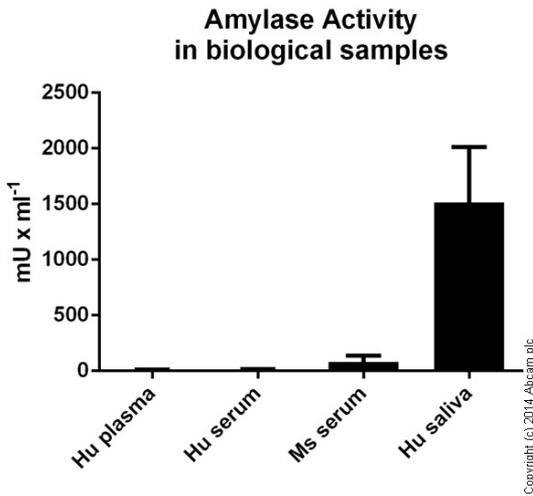
**1 Unit Amylase** = amount of amylase that cleaves ethylidene-*p*NP-G7 to generate 1.0  $\mu\text{mol}$  of nitrophenol per min at pH 7.20 at 25 °C.

## 14. TYPICAL DATA

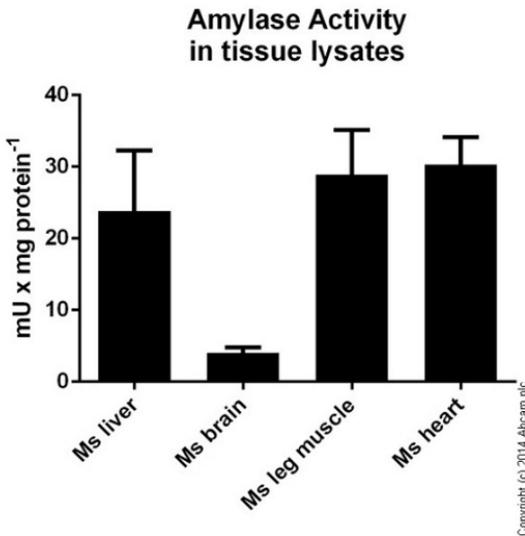
**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



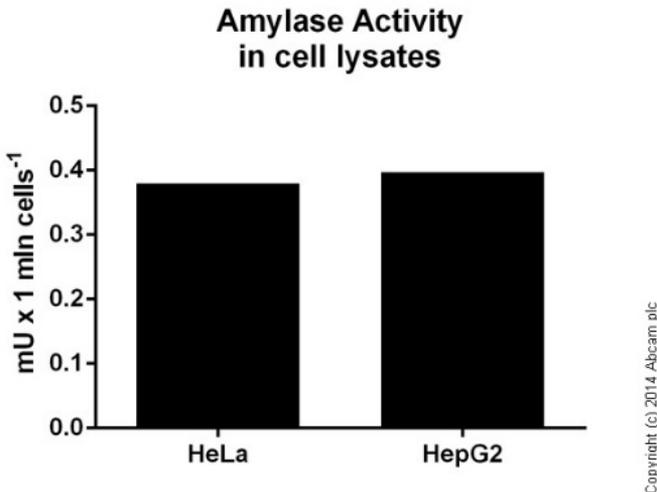
**Figure 1:** Typical Nitrophenol standard calibration curve using colorimetric reading.



**Figure 2:** Amylase Activity measured in biological fluids showing quantity (mU) per mL of tested sample after 12 min of incubation.



**Figure 3:** Amylase Activity measured in tissue lysates showing quantity (mU) per mg of protein after 12 min of incubation.



**Figure 4:** Amylase Activity measured in cell lysates showing quantity (mU) per 1 mln cells after 12 min of incubation.

## 15. QUICK ASSAY PROCEDURE

**NOTE:** This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Solubilize Amylase Positive Control, thaw the Assay Buffer, Substrate Mix and Nitrophenol standard (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50  $\mu$ L) and samples (50  $\mu$ L).
- Prepare Amylase Reaction Mix (number samples + standards + 1).

Component	Reaction Mix ( $\mu$ L)
Assay Buffer	50
Substrate Mix	50

- Add 100  $\mu$ L of Reaction Mix to standard and sample wells.
- Measure absorbance at OD=405 nm on a microplate reader in a kinetic mode at 25°C for 30 – 60 min protected from light.

## 16. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

## RESOURCES

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 $\mu\text{L}$ ) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

## 17. FAQ

### **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  $\mu\text{mol}$  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.

## 18. INTERFERENCES

## 19. NOTES

**UK, EU and ROW**

Email: [technical@abcam.com](mailto:technical@abcam.com) | Tel: +44-(0)1223-696000

**Austria**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 019-288-259

**France**

Email: [supportscientifique@abcam.com](mailto:supportscientifique@abcam.com) | Tel: 01-46-94-62-96

**Germany**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 030-896-779-154

**Spain**

Email: [soportecientifico@abcam.com](mailto:soportecientifico@abcam.com) | Tel: 911-146-554

**Switzerland**

Email: [technical@abcam.com](mailto:technical@abcam.com)

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

**US and Latin America**

Email: [us.technical@abcam.com](mailto:us.technical@abcam.com) | Tel: 888-77-ABCAM (22226)

**Canada**

Email: [ca.technical@abcam.com](mailto:ca.technical@abcam.com) | Tel: 877-749-8807

**China and Asia Pacific**

Email: [hk.technical@abcam.com](mailto:hk.technical@abcam.com) | Tel: 400 921 0189 / +86 21 2070 0500

**Japan**

Email: [technical@abcam.co.jp](mailto:technical@abcam.co.jp) | Tel: +81-(0)3-6231-0940

[www.abcam.com](http://www.abcam.com) | [www.abcam.cn](http://www.abcam.cn) | [www.abcam.co.jp](http://www.abcam.co.jp)