ab174093

Alpha-Glucosidase Activity Assay Kit (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of α-glucosidase activity in a variety of samples

View kit datasheet: www.abcam.com/ab174093

(use www.abcam.cn/ab174093 for China, or www.abcam.co.jp/ab174093 for Japan)

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5a, Last Updated 15 August 2023

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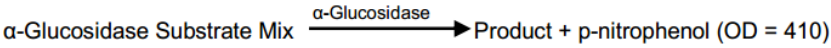
1. Overview

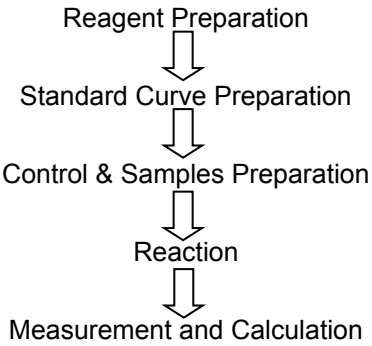
α-Glucosidase breaks down α-1,4 linked polysaccharides to glucose, which can be utilized as a source of energy. In the biotechnology industry, α-glucosidase is used to produce glucose from intermediate breakdown products of starch hydrolysis generated by enzymes such as amylase. Pompe disease, one of the 12 known glycogen storage diseases, is an autosomal recessive metabolic disorder attributed to α-glucosidase deficiency. In this disease, glycogen accumulates in the lysosomes, resulting in progressive muscle weakness, heart failure and other neurological symptoms.

In Abcam's Alpha-Glucosidase Activity Assay Kit (Colorimetric) (ab174093) α-Glucosidase hydrolyzes the Substrate Mix to release the p-nitrophenol that can be measured colorimetrically (OD = 410 nm). This is an easy, quick and high-throughput capable kit that can measure 0.1-10 mU of α-glucosidase activity in a variety of samples.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
α-Glucosidase Assay Buffer	25 mL
α-Glucosidase Substrate Mix	300 µL
α-Glucosidase Positive Control	1 vial
p-Nitrophenol Standard/p-Nitrophenol Standard (100 mM)	100 µL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96-well clear plate with flat bottom
- Temperature controlled plate reader
- Multi-channel pipette
- Distilled water

6. Reagents Preparation

1. **P-Nitrophenol Standard/p-Nitrophenol Standard (100mM):**
   Ready to use as supplied. Do not freeze/thaw. Aliquot and store at -20°C. Use within two months.

2. **α-Glucosidase Substrate Mix**:
   Ready to use as supplied. There may be significant precipitate after storage at -20°C. Brief sonication is sufficient to redissolve.

3. **α-Glucosidase Positive Control:**
   Reconstitute with 100 µL dH_2O to prepare stock solution. Aliquot Stock Solution to 10 µL/tube and store at –20°C. To prepare

working solution, dilute 10–fold more in dH_2O. Keep on ice while in use. Discard Working solution after use.

4. **α-Glucosidase Assay Buffer:**
   Warm α-Glucosidase Assay Buffer to room temperature before use.

7. Assay Protocol

1. **Sample Preparation and Positive Control:**

a) **Cells (starting material: 1 x 10^6 cells)**
   Harvest cells and spin down briefly and discard supernatant. Resuspend the cell pellet in 200 µL ice cold α-Glucosidase Assay Buffer and put on ice. Homogenize with a Douncer homogenizer (10 – 15 passes) on ice, or by pipetting up and down using a smaller tip, until efficient lysis is confirmed by viewing the cells under the microscope. Centrifuge homogenate at 12000 rpm for 5 minutes at 4°C to remove cell debris and collect the supernatant.
   Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.
   Add 10 – 50 µL test sample to the wells of a 96-well plate. If volume need is <50 µL, bring it up to 50 µL with α-Glucosidase Assay Buffer.

b) **Tissue Sample (starting material: 10mg tissue)**

Cut tissue in small pieces, add 200 µL ice cold α-Glucosidase Assay Buffer and put on ice. Homogenize using a Douncer homogenizer (10 – 15 passes) on ice, until efficient lysis is confirmed, by viewing the cells under the microscope. Spin down the samples and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 10 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with α-Glucosidase Assay Buffer.

c) Liquid Samples (serum and saliva)

Briefly centrifuge at 12000 rpm for 5 min. at 4°C. Collect supernatant.

Add 10 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with α-Glucosidase Assay Buffer.

NOTE:

For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

Positive Control:

Add 2 – 10 µL of α-Glucosidase Positive Control working solution into well(s). Adjust final volume to 50 µL with α-Glucosidase Assay Buffer.

2. Standard Curve Preparation:

a) Prepare a 10 mM p-Nitrophenol Standard by adding 10 µL of 100 mM p-Nitrophenol Standard to 90 µL of α-Glucosidase Assay Buffer. Mix well by pipetting up and down.

b) Using the 10 mM p-Nitrophenol standard, prepare standard curve as follows, in a microplate or microcentrifuge tubes:

p-NP 10mM amount (µL)	**Glucosidase assay buffer (µL)**	**Amount in well**	**END CONCENTRATION p-NITROPHENOL IN WELL**
0	300	100 µl	0 nmol/well
6	294	100 µl	20 nmol/well
12	288	100 µl	40 nmol/well
18	282	100 µl	60 nmol/well
24	276	100 µl	80 nmol/well
30	270	100 µl	100 nmol/well

Add 100 µl of each standard dilution into a 96-well plate to set up standard.

Each dilution has enough amount of standard to set up 2 duplicates x 100 µl/well.

c) Read absorbance at λ = 410 nm and keep 96-well plate in the plate reader to bring to temperature while preparing reaction mix.

3. Reaction and Detection:

a) Reaction mix: Prepare Reaction Mix for each reaction:

	Reaction Mix
α-Glucosidase Assay Buffer	47 µL
α-Glucosidase Substrate Mix	3 µL

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

	Reaction Mix
α-Gluc Assay Buffer	47 µL x (Nb samples +positive control + 1)
α-Gluc Substrate Mix	3 µL x (Nb samples + positive control + 1)

b) Mix & add 50 µL Reaction Mix to Positive Control and sample wells and mix well.

c) Start to read OD410nm immediately in kinetic mode. Reading should continue for 15 – 60 minutes, depending on the amount of enzyme present in samples (see Fig 2A).

8. Data Analysis

Calculations:

a) Correct background by subtracting the value derived from the zero standard from all sample readings

b) Plot Standard Curve for p-Nitrophenol.

c) For samples, select the linear portion of the kinetic curve for activity analysis. Excel has a simple function, which calculates slope for a series of data points.

d) Determine the rate of change of OD/min for the unknown samples. Use Standard Curve to convert OD/minute to nmole/minute

Concentration of α-glucosidase in samples = (Sa/Ss)/ V
= mU/µL = U/mL

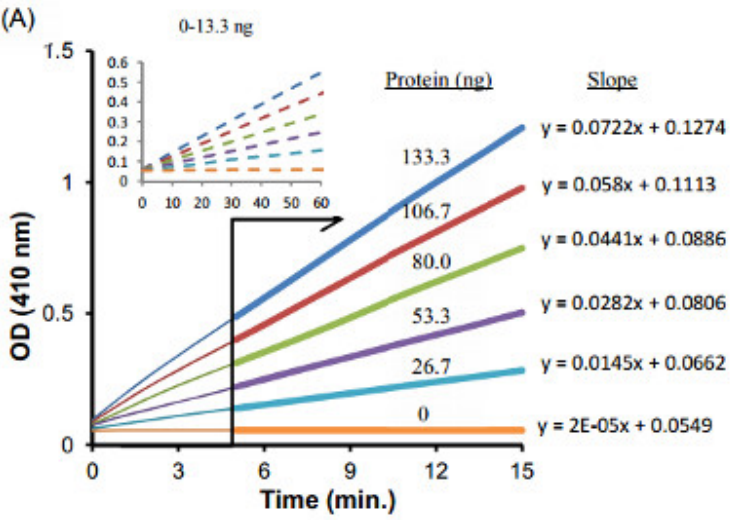
Where:

Sa = slope of the enzyme activity (OD/min) in the sample well

Ss = slope of Standard Curve (OD/nmol)

V = volume of sample added in the well (µL)

(A)


0-13.3 ng
Protein (ng)
Slope
133.3
106.7
80.0
53.3
26.7
0
y = 0.0722x + 0.1274
y = 0.058x + 0.1113
y = 0.0441x + 0.0886
y = 0.0282x + 0.0806
y = 0.0145x + 0.0662
y = 2E-05x + 0.0549
OD (410 nm)
Time (min.)

(B)

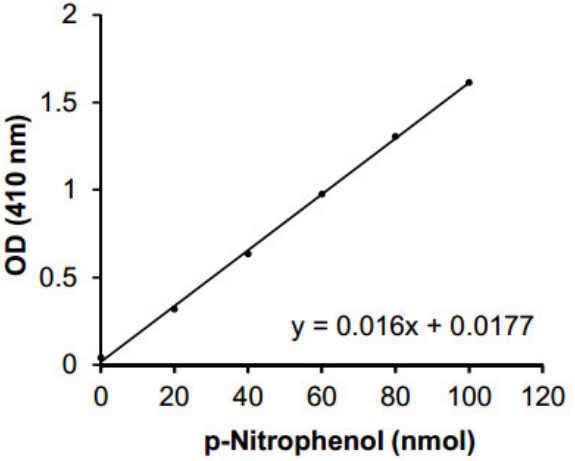

y = 0.016x + 0.0177
OD (410 nm)
p-Nitrophenol (nmol)

Figure 2. : α-Glucosidase kinetic assay **(A)** Kinetic profile of various amounts (0, 2, 4, 6, 8 & 10 mU) of α-glucosidase run at 25°C under this protocol. Inset: Results for 0-0.2-0.4-0.6-0.8-1.0 mU of α-glucosidase. Data points after 5 minutes were used to determine slope. **(B)** p-Nitrophenol Standard Curve

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:
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