Technical Bulletin

α-Glucosidase Activity Colorimetric Assay Kit

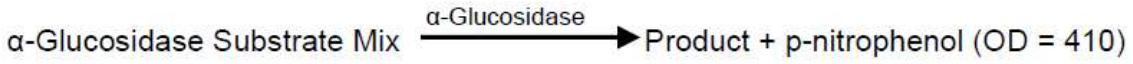
Catalog Number MAK385

Product Description

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 the α-Glucosidase Activity Colorimetric Assay Kit, α-glucosidase hydrolyzes the substrate mix to release p-nitrophenol that can be measured colorimetrically at 410 nm. This is an easy and quick assay suitable for high-throughput applications with a linear range of 0.1-10 mU of α-glucosidase.

The kit is suitable for the determination of α-glucosidase activity in a variety of samples including serum, saliva, and tissue and cell culture.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- α-Glucosidase Assay Buffer 25 mL
  Catalog Number MAK385A
- α-Glucosidase Substrate Mix 300 µL
  Catalog Number MAK385B
- α-Glucosidase Positive Control 1 vial
  Catalog Number MAK385C
- p-Nitrophenol Standard (100 mM) 100 µL
  Catalog Number MAK385D

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader capable of temperature control at 25 °C
- Refrigerated microcentrifuge capable of RCF ≥12,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

α-Glucosidase Assay Buffer: Ready to use as supplied. Warm α-Glucosidase Assay Buffer to room temperature prior to use. Chill an appropriate amount of α-Glucosidase Assay Buffer for use in Sample Preparation.

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.

α-Glucosidase Positive Control: Reconstitute with 100 µL of purified water to prepare stock solution. Aliquot Stock Solution to 10 µL per tube and store at -20 °C. Do not freeze/thaw. Use within two months of reconstitution.

Procedure

Prewarm plate reader to 25 °C.

Standard Curve Preparation

1. Prepare a 10 mM p-nitrophenol (pNP) solution by diluting 10 µL of p-Nitrophenol Standard (100 mM) with 90 µL of α-Glucosidase Assay Buffer, mix well. Prepare p-Nitrophenol Standards according to Table 1. Mix well.

Table 1.

Preparation of p-Nitrophenol (pNP) Standards

Well	10 mM pNP Standard	α-Gluco-sidase Assay Buffer	p-Nitro-phenol (nmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	20
3	4 µL	96 µL	40
4	6 µL	94 µL	60
5	8 µL	92 µL	80
6	10 µL	90 µL	100

Read absorbance of all standards at 410 nm (A_{410}) and keep 96-well plate in plate reader to bring to temperature (25 °C) while preparing reaction mix.

Sample Preparation

1. Rapidly homogenize tissue (10 mg) or cells (1 x 10^6 cells) with 200 µL of ice-cold α-Glucosidase Assay Buffer on ice.
2. Centrifuge at 12,000 × *g* for 5 minutes at 4 °C.
3. Collect the supernatant.
4. For serum and saliva, centrifuge at 12,000 × *g* for 5 minutes at 4 °C. Collect supernatant.
5. Add 10-50 µL of unknown samples into 96-well plate. Adjust the total volume to 50 µL/well with α-Glucosidase Assay Buffer. For unknown samples, perform a pilot experiment by testing several dilutions to ensure the readings are within the Standard Curve range.

Positive Control

1. Prepare Working Solution by adding a 10 µL aliquot of the reconstituted α-Glucosidase Positive Control solution to 90 µL of purified water. Keep on ice while in use and discard remaining working solution after use.
2. Add 2-10 µL of α-Glucosidase Positive Control Working Solution into well(s). Adjust total volume to 50 µL with α-Glucosidase Assay Buffer.

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed. For each Sample and Positive Control well, prepare 50 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
α-Glucosidase Assay Buffer	47 µL
α-Glucosidase Substrate Mix	3 µL

2. Add 50 µL of Reaction Mix to each Sample and Positive Control well. Mix well.

Measurement

Start to read at 410 nm (A_{410}) immediately in kinetic mode at 25 °C. Reading should continue for between 15-60 minutes, depending upon the amount of enzyme in Samples.

Results

1. Plot Standard Curve for p-Nitrophenol and calculate the slope for the curve.
2. For Samples, select the linear portion of the kinetic curve for activity analysis.
3. Determine the rate of change of A_{410}/minute for the unknown samples.
4. Use Standard Curve to convert OD_{410}/minute to nmole/minute.

α-Glucosidase Activity (mU/µL = U/mL) =

$$(S_a/S_s)/ V$$

where:

S_a = Slope of the enzyme activity (A_{410}/minute) in the sample well

S_s = Slope of Standard Curve (A_{410}/nmol)

V = Sample volume added into the reaction well (µL)

Sample α-Glucosidase activity can also be expressed in U/mg protein.

Unit Definition: One unit of α-Glucosidase is the amount of enzyme that generates 1.0 µmol of p-Nitrophenol per minute at pH 7.4 at 25 °C.

Figure 1.
Typical p-Nitrophenol Standard Curve

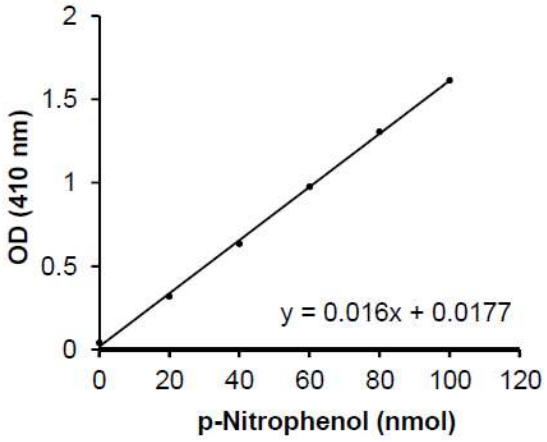
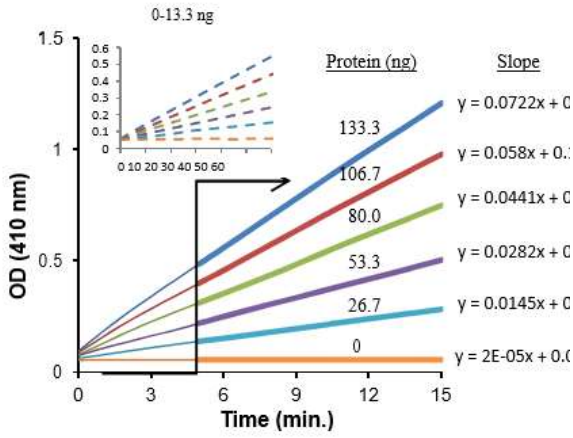


Figure 2.
Kinetic profile of various amounts (0, 2, 4, 6, 8 and 10 mU) of α-glucosidase run at 25 °C using the kit protocol. Inset: Results for 0-0.2-0.4-0.6-0.8-1.0 mU of α-glucosidase. Data points after 5 minutes were used to determine slope.

In this example, 133.3 ng of enzyme gave a slope of 0.722 A_{410}/minute. From the slope of the standard curve, 1 nmol of p-nitrophenol gives 0.016 A_{410}.
S_a/S_s = (0.0722/0.016) = 4.5125 nmol/min.
Sample α-Glucosidase activity = (4.5125 mU / 0.1333µg) = 33.84 mU/µg or 33.84 U/mg protein.



Frequently Asked Questions:

Does the substrate mix specifically work with only α-glucosidase or can it cross-react with α-amylase?

α-Glucosidase is a type of amylase that hydrolyzes 1,4-α-glucoside bonds. The substrate is specific for α-glucosidase and does not interact with α-amylase.

Will samples in RIPA buffer work with this kit?

The α-Glucosidase Assay Buffer provided in the kit is recommended for tissue homogenization. RIPA buffer typically contains SDS, which should be avoided in this protocol due to potential adverse effects to enzymes in the kit.

Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can be used provided:

- Samples were frozen immediately after isolation
- Samples have not undergone multiple freeze/thaw cycles
- Samples have been frozen for a relatively short time.

It is recommended to aliquot samples prior to freezing to minimize free/thaw cycles.

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