Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

α-Glucosidase Activity Colorimetric Assay Kit

rev. 4/13

(Catalog # K690-100; 100 assays; Store at -20°C)

I. Introduction:

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 BioVision's α-Glucosidase Activity Colorimetric Assay Kit, 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.

$$\text{α-Glucosidase Substrate Mix} \xrightarrow{\text{α-Glucosidase}} \text{Product + p-nitrophenol (OD = 410)}$$

II. Application:

- Measurement of α-Glucosidase activity in biological samples
- Screening α-Glucosidase inhibitors

III. Sample Type:

- Serum
- Saliva
- Tissue
- Cell Culture

IV. Kit Contents:

Components	K690-100	Cap Code	Part Number
α-Glucosidase Assay Buffer	25 ml	WM	K690-100-1
α-Glucosidase Substrate Mix	300 µl	Amber	K690-100-2
α-Glucosidase Positive Control	1 Vial	Blue	K690-100-3
p-Nitrophenol Standard (100 mM)	100 µl	Yellow	K690-100-4

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm α-Glucosidase Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **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 dH_2O to prepare stock solution. Aliquot Stock Solution to 10 µl/tube and store at –20°C. Do not freeze/thaw. Use within two months. To prepare working solution, dilute 10–fold more. Keep on ice while in use. Discard Working solution after use.

VIII. α-Glucosidase Activity Assay Protocol:

1. **Prewarm:** Plate reader to 25°C.
2. **Standard Curve:** Dilute p-nitrophenol to 10 mM with α-Glucosidase Assay Buffer by adding 10 µl of 100 mM p-Nitrophenol Standard to 90 µl of α-Glucosidase Assay Buffer. Add 0, 2, 4, 6, 8 &10 µl of diluted 10 mM p-Nitrophenol Standard into a series of wells in a 96-well plate. Adjust volume to 100 µl/well with α-Glucosidase Assay Buffer. Read absorbance at 410 nm and keep 96-well plate in plate reader to bring to temperature while preparing reaction mix.
3. **Samples:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold α-Glucosidase Assay Buffer on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. For serum & saliva, briefly centrifuge at 12000 rpm for 5 min. at 4°C. Collect supernatant. Add 10-50 µl of unknown samples into 96-well plate. Adjust the volume to 50 µl/well.

   Note:

   We recommend making several dilutions of your samples to ensure that the values for unknowns fall within the limits of the Standard Curve.
4. **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.
5. **Reaction Mix:** Mix enough reagents for the number of assays (samples & positive control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

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

Mix & add 50 µl Reaction Mix to Positive Control & sample wells and mix well.

6. **Measurement:** Start to read OD immediately in kinetic mode at 410 nm. Reading should continue for between 15-60 minutes, depending upon the amount of enzyme in samples (Fig 1A).
7. **Calculation:** Plot Standard Curve for p-Nitrophenol. 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. 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** = the slope of the enzyme activity (OD/min) in the sample well.
Ss = slope of Standard Curve (OD/nmol)
V = sample volume added to the 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 min at pH 7.4 at 25^{0}C.

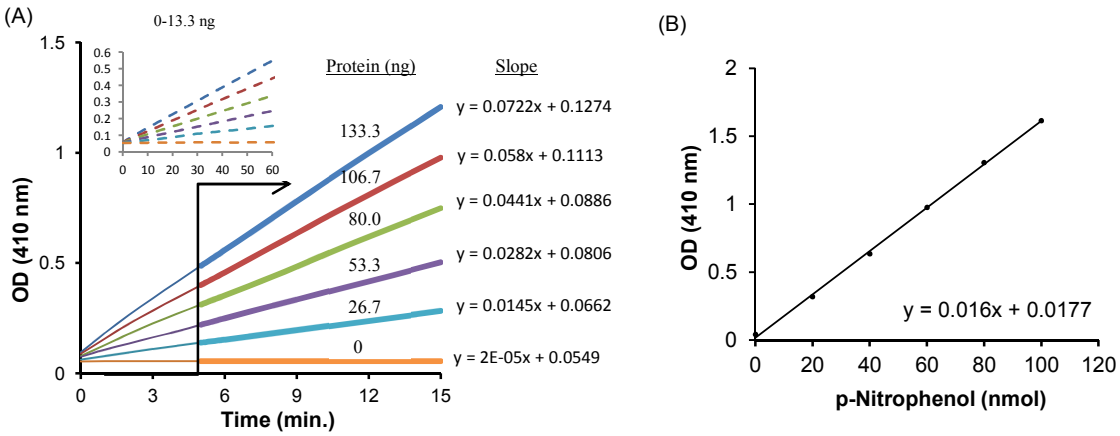

Figure: α-Glucosidase kinetic assay. (A) Kinetic profile of various amounts (0, 2, 4, 6, 8 & 10 mU) of α-glucosidase run at 25°C under this protocol. Inset: Results for 0-0.2-0.4-0.6-0.8-1.0 mU of α-glucosidase. Data points after 5 minutes were used to determine slope. (B) p-Nitrophenol Standard Curve.

In this example, 133.3 ng of enzyme gave a slope of 0.722 OD/minute. From the slope of the standard curve, we see that 1 nmol of p-nitrophenol gives 0.016 OD. Sa/Ss = (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.

IX. RELATED PRODUCTS:

Amylase Activity Colorimetric Assay Kit
Glucose Colorimetric/Fluorometric Assay kit
PicoProbe™ Glucose Fluorometric Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Glucose-1-Phosphate Colorimetric Assay Kit
Glucose Uptake Colorimetric Assay Kit
Galactose Colorimetric/Fluorometric Assay Kit
Glycogen Colorimetric/Fluorometric Assay Kit
Hexokinase Colorimetric Assay Kit
Maltose & Glucose Colorimetric/Fluorometric Assay Kit
PEP Colorimetric/Fluorometric Assay Kit
Phosphoglucomutase Colorimetric Assay Kit
Pyruvate Colorimetric/Fluorometric Assay Kit
Maltose and Glucose Assay Kit
NADP/NADPH Quantification Kit
Total Carbohydrate Assay Kit
Starch Colorimetric/Fluorometric Assay Kit
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit
Glucose Colorimetric Assay Kit II
Glucose-6-Phosphate Dehydrogenase Assay Kit
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit
Glucose Uptake Fluorometric Assay Kit
Galactose & Lactose Colorimetric/Fluorometric Assay Kit
Glycogen Colorimetric Assay Kit II
Maltose Colorimetric/Fluorometric Assay Kit
PicoProbe™ Lactate Fluorometric Assay Kit
Phosphofructokinase (PFK) Activity Colorimetric Assay Kit
Phosphoglucose Isomerase Colorimetric Assay Kit
Pyruvate Kinase Activity Colorimetric Assay Kit
NAD/NADH Quantification Kit
PicoProbe™ NADH Fluorometric Assay Kit
ATP Colorimetric/Fluorometric Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.