Alpha Galactosidase (α-Gal) Activity Assay Kit (Fluorometric)

(Catalog # BN00660; 100 assays; Store at -20°C)

I. Introduction:

Alpha-Galactosidase (α-Gal; EC 3.2.1.22) hydrolyzes alpha-galactosyl moieties found in glycolipids and glycoproteins. In mammals, α-Gal hydrolyzes poly- and oligosaccharides commonly found in dietary sources that are difficult to digest. Therefore, α-Gal is used in dietary supplements that help to reduce the production of intestinal gases due to consumption of certain foods. It is known total α-Gal activity is due to two major isozymes with unique, yet different thermostability profiles. Alpha-Galactosidase A, is thermolabile and represents approximately 90% of total α-Gal activity. Fabry Disease, a lysosomal disease disorder, is characterized by mutations in alpha-Galactosidase A. These mutations cause abnormal accumulation of glycosphingolipids in lysosomes. Assay Genie's Alpha Galactosidase Activity Assay Kit provides a simple, rapid way to monitor total α-Gal activity in wide variety of biological samples. In this kit, α-Gal cleaves a synthetic specific substrate releasing a fluorophore, which can be easily quantified (Ex/Em= 360/445 nm). The assay is specific, sensitive and can detect as low as 0.1 µU of α-Galactosidase activity.

α-Gal Substrate $\xrightarrow{\text{Alpha Galactosidase}}$ Cleaved Substrate + Fluorescent Product (Ex/Em= 360/445 nm)

II. Applications:

- Measurement of α-Galactosidase activity in various samples

III. Sample Type:

- Tissue Homogenates: kidney, etc.
- Cell Lysates: U937, etc.
- Biological fluids: Saliva, etc.

IV. Kit Contents:

Components	BN00660	Cap Code	Part Number
α-Gal Assay Buffer	25 ml	NM	BN00660-1
α-Gal Stop Buffer	25 ml	WM	BN00660-2
α-Gal Substrate	220 µl	Blue	BN00660-3
4-Methylumbelliferone Standard	35 µl	Yellow	BN00660-4
α-Gal Positive Control	1 vial	Green	BN00660-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- 96-well white plate with flat bottom is preferred for this assay. 96-well clear plate can also be used.
- Dounce Tissue Homogenizer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **α-Gal Assay Buffer and Stop Buffer:** Store at 4 ºC or -20 ºC. Bring to 37 °C before use.
- **α-Gal Substrate:** Light sensitive. Thaw at room temperature. Store at -20 ºC.
- **4-Methylumbelliferone Standard (5 mM):** Light sensitive. Thaw at room temperature. Store at -20 ºC.
- **α-Gal Positive Control:** Reconstitute with 20 µl α-Gal Assay Buffer and mix thoroughly. Store at -20 ºC. Keep on ice while in use. Use within two months.

VII. α-Gal Activity Assay Protocol:

1. **Sample Preparation: For tissue and cells:** Homogenize tissue (10 mg) or pelleted cells (~5 x 10^5) with 100 µl ice-cold α-Gal Assay Buffer and keep on ice for 10 min. Centrifuge samples at 12,000 x *g* at 4 ºC for 10 min. and collect the supernatant. Dilute the supernatant 10-20 fold in α-Gal Assay Buffer. Add 2-10 µl of diluted samples into a 96-well plate that will be designated as Sample(s). **For biological fluids:** Undiluted fluids can be added directly to the well. Add 2-10 µl of samples into well(s) in a 96-well plate that will be designated as Samples. **For Reagent Background Control**: add same volume of α-Gal Assay Buffer in parallel well(s). **For Positive Control:** dilute reconstituted α-Gal Positive Control 1:10 fold with α-Gal Assay Buffer prior to the assay and add 2-6 µl of diluted α-Gal Positive Control into desired wells(s). Adjust the volume of Positive Control, Sample(s), and Reagent Background Control to **40 µl/well** with α-Gal Assay Buffer.

   Note:

   a. We suggest using 3-5 different volumes of the samples per well to ensure the readings are within the standard curve range and the progress curve rates are within the linear range.

   b. Do not store unused diluted α-Gal Positive Control.

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 5 mM 4-MU to 490 µl α-Gal Assay Buffer in amber tube. Further dilute the 100 µM Standard solution 5-fold by adding 20 µl of 100 µM 4-MU to 80 µl α-Gal Assay Buffer to generate 20 µM 4-MU Standard. Add 0, 2, 4, 6, 8, 10 µl of 20 µM 4-MU standard into a series of wells to generate 0, 40, 80, 120, 160, 200 pmol/well of 4-MU Standard respectively. Adjust the volume to **60 µl/well** with α-Gal Assay Buffer.

Note: Equilibrate the α-Gal Assay Buffer to 37 ºC prior to the assay.

3. **Substrate Hydrolysis:** Prepare sufficient volume of 10-fold dilution of α-Gal Substrate (i.e. Dilute 4 µl of α-Gal stock Substrate with 36 µl of α-Gal Assay Buffer), vortex briefly. Add 20 µl of diluted α-Gal Substrate to each well containing the test Sample(s), Positive Control and Reagent Background Control. *The total volume in each well (i.e. Samples, Positive Control and Reagent Background Control) should be 60 µl)*. **Mix well and incubate at 37 °C for 2 hours, avoid light**. After incubation, add 200 µl of α-Gal Stop Buffer to each well containing Sample(s), Positive Control, Reagent Background Control and Standards. Mix well.

   Note:

   a. Equilibrate the α-Gal Stop Buffer to 37 ºC prior to the assay.

   b. Standards can be prepared at the end of the incubation time, and measured in end-point mode.

4. **Measurement:** Measure fluorescence intensity (Ex/Em= 360/445 nm) at 37°C using an end-point setting.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve; subtract the Reagent Background Control reading from all Sample readings. Apply sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (**B**, in pmol) and calculate the activity of α-Galactosidase activity in the sample as:

Specific Sample α-Galactosidase Activity = B/ (2 x V x P) x D (pmol/h/mg ≡ 0.0167 µU/mg)

Where: **B** = 4-MU amount in sample well from Standard Curve (pmol)
2 = Reaction time (hour)
V = Sample volume added into the reaction well (ml)
P = Initial Sample Concentration in mg-protein/ml (mgP/ml)
D = Sample Dilution Factor

1 pmol/h= 0.0167 pmol/min ≡ 0.0167 µU

Unit Definition: One unit of α-Galactosidase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone per min., at pH 4.5 at 37 °C.

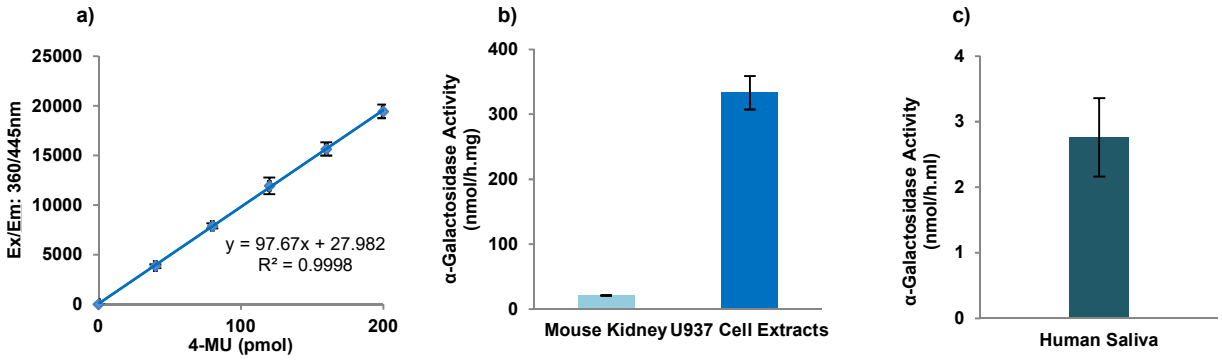

Figure: (a) 4-Methylumbelliferon Standard Curve. Results are from multiple experiments. (b) α-Galactosidase Activity in Mouse Kidney Tissue Extracts (1 µg protein) and U937 Cell Lysates (0.2 µg protein). (c) Measurement of α-Galactosidase Activity in undiluted Human Pooled Saliva (5 µl). All assays were performed following kit protocol.

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