Technical Bulletin

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

Catalog Number MAK390

Product Description

Alpha-Galactosidase (α-Gal) hydrolyzes α-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.

The Alpha Galactosidase (α-Gal) Activity Assay Kit provides a simple, rapid way to monitor total α-Gal activity in a wide variety of biological samples. In this kit, α-Gal cleaves a synthetic specific substrate, releasing a fluorophore which can be easily quantified (λ_{Ex} = 360 nm/λ_{Em} = 445 nm). The assay is specific (β-galactosidase activity is not detected), sensitive, and capable of detecting as little as 0.1 μU of α-Galactosidase activity.

The kit is suitable for the detection of α-Galactosidase activity in tissue homogenates (kidney, etc.), cell lysates (U937, etc.) and biological fluids (saliva, etc.).

Components

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

- α-Gal Assay Buffer 25 mL
  Catalog Number MAK390A
- α-Gal Stop Buffer 25 mL
  Catalog Number MAK390B
- α-Gal Substrate 220 μL
  Catalog Number MAK390C
- 4-Methylumbelliferone Standard 35 μL
  Catalog Number MAK390D
- α-Gal Positive Control 1 vial
  Catalog Number MAK390E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Flat bottom, white or clear (white is preferred for this assay) 96-well plate. Cell culture or tissue culture treated plates are **not** recommended.
- 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. Upon opening, use kit within 2 months.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

α-Gal Assay Buffer and Stop Buffer: Store at 2-8 ºC or -20 °C. Warm to 37 °C prior to use. Chill an appropriate amount of α-Gal Assay Buffer for use in Sample Preparation

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 of α-Gal Assay Buffer and mix thoroughly. Store at -20 ºC. Keep on ice while in use. Use within two months.

Procedure

Sample Preparation

Tissue and cells

1. Homogenize tissue (10 mg) or pelleted cells (~5 x 10^5 cells) with 100 µL of ice-cold α-Gal Assay Buffer using a Dounce homogenizer and keep on ice for 10 minutes.
2. Centrifuge samples at 12,000 × *g* at 4 ºC for 10 minutes and collect the supernatant.
3. Dilute the supernatant 10-20-fold in α-Gal Assay Buffer.
4. Add 2-10 µL of diluted samples into a 96-well plate that will be designated as Sample(s). It is recommended to use 3-5 different volumes of each sample in separate wells to ensure the readings are within the standard curve range and the progress curve rates are within the linear range.
5. Adjust the total volume in each well to 40 µL with α-Gal Assay Buffer.

Biological Fluids

1. 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. It is recommended to use 3-5 different volumes of each sample in separate wells to ensure the readings are within the standard curve range and the progress curve rates are within the linear range.
2. Adjust the total volume in each well to 40 µL with α-Gal Assay Buffer.

Reagent Background Control

In parallel well(s), prepare Reagent Background Control by adding 40 µL/well of α-Gal Assay Buffer.

Positive Control

1. Dilute reconstituted α-Gal Positive Control 1:10 with α-Gal Assay Buffer prior to the assay and add 2-6 µL of diluted α-Gal Positive Control into desired wells(s).
2. Adjust the total volume to 40 µL/well with α-Gal Assay Buffer.

Note: Do not store unused diluted α-Gal Positive Control.

Standard Curve Preparation

Note: Standards can be prepared at the end of the incubation time (Step 2 of Assay Procedure) and measured in end-point mode.

1. Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µL of 5 mM 4-MU to 490 µL of α-Gal Assay Buffer in an amber tube.

2. Prepare a 20 µM 4-MU Standard by diluting the 100 µM Standard solution from Step 1 1:5 by adding 20 µL of 100 µM 4-MU to 80 µL of α-Gal Assay Buffer.
3. Prepare 4-MU Standards according to Table 1. Mix well.

Table 1.
Preparation of 4-MU Standards

Well	20 µM 4-MU Standard	α-Gal Assay Buffer	4-MU (pmol/well)
1	0 µL	50 µL	0
2	2 µL	58 µL	40
3	4 µL	56 µL	80
4	6 µL	54 µL	120
5	8 µL	52 µL	160
6	10 µL	50 µL	200

α-Gal Substrate Working Solution

For each test Sample(s), Positive Control and Reagent Background Control well, prepare 20 µL of α-Gal Substrate Working Solution according to Table 2. Mix well.

Table 2.
Preparation of α-Gal Substrate Working Solution

Reagent	Volume
α-Gal Substrate	2 µL
α-Gal Assay Buffer (pre-warmed to 37 °C)	18 µL

Assay Procedure

1. Add 20 µL of α-Gal Substrate Working Solution 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. **Do not add α-Gal Substrate Working Solution to Standard wells.**
2. Mix well and incubate at 37 °C for 2 hours, protected from light.
3. 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.

Measurement

Measure fluorescence intensity (RFU) (λ_{Ex} = 360 nm/λ_{Em} = 445 nm) at 37 °C using end-point mode.

Results

1. Subtract 0 Standard reading (RFU) from all Standard readings.
2. Plot the 4-MU Standard Curve.
3. Subtract the Reagent Background Control reading (RFU) from all Sample readings (RFU).
4. Apply the corrected Sample RFU to the 4-MU Standard Curve to obtain the corresponding pmol of product formed (B). Calculate the activity of α-Galactosidase activity in the sample using the following equation:

α-Galactosidase Activity (pmol/h/mg ≡ 0.0167 µU/mg) =

$$\frac{B}{2 \times V \times P} \times D$$

where:

B = 4-MU amount in sample well from Standard Curve (pmol)

2 = Reaction time (hours)

V = Volume of the sample used in the reaction (in mL)

P = Initial Sample Concentration (in mg protein/mL)

D = Sample Dilution Factor (if applicable)

Note: 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 minute at pH 4.5 at 37 °C.

Figure 1.
Typical 4-Methylumbelliferone Standard Curve

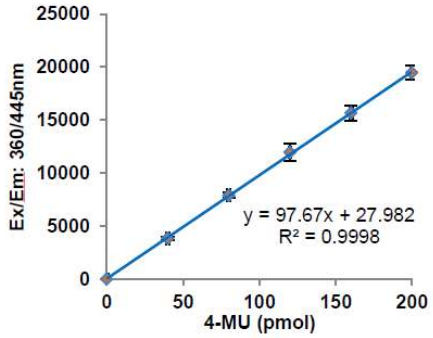


Figure 2.
α-Galactosidase Activity in Mouse Kidney Tissue Extracts (1 μg protein) and U937 Cell Lysates (0.2 μg protein) measured following the kit protocol.

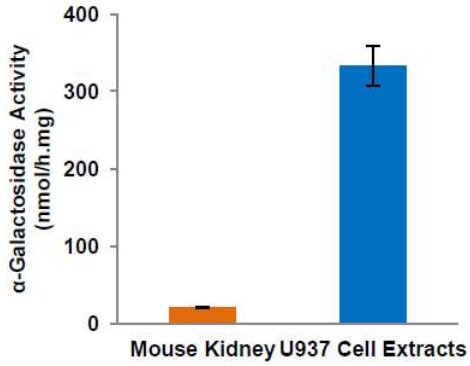
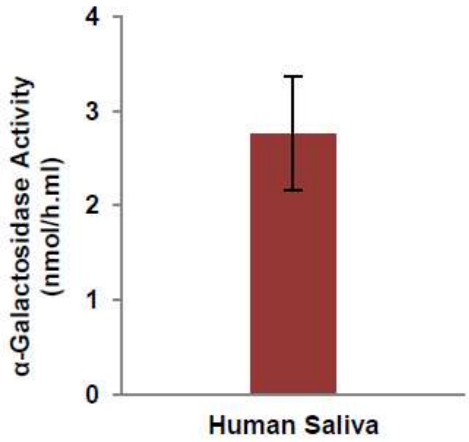


Figure 3.
α-Galactosidase Activity in undiluted Human Pooled Saliva (5 μL) measured following the kit protocol.



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