MA-0142

α-Galactosidase Activity Assay

(100 wells, Fluorometric, Ex/Em = 360/445 nm, Store at -20°C)

Introduction:

α-Galactosidase hydrolyzes α-galactosyl residues found in glycolipids, polysaccharides and glycoproteins. In mammals, α-Galactosidase is produced by the pancreas and released into the small intestine, where it hydrolyzes galactosides from dietary sources. It is also found in dietary supplements to reduce the production of intestinal gases. α-Galactosidase comes as two primary isoforms. α-Galactosidase A accounts for ~90% of α-Galactosidase activity and in Fabry Disease, is mutated and dysfunctional resulting in abnormal accumulation of lysosomal glycosphingolipids. AkrivisBio's α-Galactosidase Activity Assay is a simple, sensitive means of monitoring α-Galactosidase activity in biological samples. In the assay a synthetic substrate is acted on by α-Galactosidase releasing a fluorophore. The assay LOD is below 0.1 µU of α-Galactosidase activity.

Assay Principle:

1 - α-Galactosidase cleaves a synthetic substrate (4-MU-α-D-galactopyranoside) releasing Methylumbelliferone.
2 – Add stop solution to wells to shift pH to enhance fluorescence yield.
2 – Reaction progress is monitored by fluorescence using excitation 360 nm, emission 445 nm.

Assay Components:

Assay Buffer	25 ml	NM	MA-0142-A
Stop Buffer/Enhancer	25 ml	WM	MA-0142-B
Artificial Substrate	200 µl	Amber /Blue	MA-0142-C
4-Methylumbelliferone Standard	400 µl	Amber/Yellow	MA-0142-D
α-Galactosidase Positive Control	lyoph	Green	MA-0142-E

User Supplied Reagents and Equipment:

- 96-well white or black plate with flat bottom

Storage Handling Considerations:

Store unopened kit at -20°C. Centrifuge small vials briefly prior to opening.
Assay Buffer; Stop Buffer: Ready to use as supplied. Store at 4°C.
Artificial Substrate: Store at -20°C. Light sensitive.
4-Methylumbelliferone Standard: 20 µM. Ready to use as supplied, Store at -20°C. Light sensitive.
α-Galactosidase Positive Control: Reconstitute with 20 µl Assay Buffer. Store at -20°C. Keep on ice while in use.

Assay Protocol:

1. Warm Assay Buffer to 37°C before starting assay.

2. Standard Curve: Standard is supplied at 20 µM. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl of 20 µM 4-MU standard to a series of wells in a 96 well plate, giving 0 – 40 – 80 – 120 – 160 – 200 pmol of 4-MU Standard. Adjust all well volumes to 60 µl with Assay Buffer.

3. Test Samples: Homogenize tissue (10 mg) or cells (5 x 10^5 – 1 x 10^6) with 100 µl ice-cold Assay Buffer. Centrifuge samples at 12,000 x *g* at 4 ºC for 10 minutes. Transfer the clear supernatant to a fresh tube and dilute 10-fold with Assay Buffer. Transfer 2-10 µl of diluted samples to a 96-well plate. 2-10 µl of undiluted fluids can be added directly to the plate.

4. Reagent Background Control: Add 2-10 µl of Assay Buffer **to a** well.

5. Positive Control: Dilute1-2 µl reconstituted Positive Control 10X with Assay Buffer then transfer 5 µl of diluted Positive Control to desired wells. Adjust all well volumes (Test Samples, Reagent Background Control, Positive Control) to 40 µl with Assay Buffer. Preincubate the plate at 37°C while preparing substrate.

6. Initiate Reaction: The substrate will be diluted 10X with Assay Buffer before adding to the wells. Each Test Sample, Reagent Background Control and Positive Control well will require 20 µl of diluted substrate (2 µl Substrate + 18 µl Assay Buffer). Prepare sufficient material for the number of wells to be run. Warm diluted substrate to 37°C. Transfer 20 µl of diluted Substrate to all Test Sample, Positive Control and Reagent Background Control wells. Make only as much diluted substrate as needed. Diluted substrate is unstable and should be discarded if not used.

7. Mix well and incubate at 37 °C for 2 hours, in the dark. The reaction can be monitored using the settings in step 9 below without the addition of stop solution but with about 5% of the sensitivity.

8. Stop Reaction: After 2 hours, add 200 µl of Stop Buffer/Enhancer to all wells (Test Sample, Positive Control, Background Control, Standards) and mix.
Note: Standards can be prepared at the end of the incubation time, and measured in end-point mode.

9. Measurement: Measure fluorescence intensity using Excitation 360 nm, Emission 445 nm at 37°C.

10. Typical Results:

11. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the Standard Curve. Determine the slope of the Standard Curve. This defines the sensitivity of the system. Subtract the Reagent Background Control reading from all Test Sample readings. Divide the background corrected test sample readings by the slope of the standard curve to get the pmoles of 4-MU generated in 4 hours for the test samples. Convert the enzyme activity in the wells back to enzyme activity in the original sample as follows:

A. Divide the pmoles in the test well by the volume added to the well = pmoles generated/µl sample in 2 hours (or whatever incubation time was used.)
B. Multiply the pmoles generated/µl sample by the total volume of sample transferred after centrifugation in step 3 above.
C. Multiply by 10 to correct for the dilution in step 3 = total pmoles generated for each sample in 2 hours
D. Divide by the mg tissue used or the # of cells, etc. = pmoles generated in 2 hours / mg tissue (or /10^6 cells, etc.)

FOR RESEARCH USE ONLY! Not to be used on humans

48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com