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# ab239716 Alpha Galactosidase Activity Assay Kit

For the measurement of total alpha galactosidase activity in a wide variety of biological samples.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

# 1. Overview

Alpha Galactosidase Activity Assay Kit (ab239716) provides a simple, rapid way to monitor total alpha galactosidase(a-Gal) activity in a wide variety of biological samples. In this kit, a-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 a-Galactosidase activity. This kit does not detect beta galactosidase activity.

## 2. Protocol Summary

Prepare sample, standards and controls.

Add 20 µL of diluted a-Gal Substrate to each well. Mix well.

Incubate at 37°C for 2 h protected from light.

Add 200 µL of Stop Solution V to each well. Mix well.

Measure fluorescence intesity (Ex/Em = 360/445 nm) at 37°C using an end-point setting.

## 3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

## 4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

| Item | Quantity | Storage condition |
|---|---|---|
| Assay Buffer 25 | 25 mL | -20°C |
| Stop Solution V | 25 mL | -20°C |
| a-Gal Substrate | 220 µL | -20°C |
| 4-Methylumbelliferone Standard | 35 µL | -20°C |
| alpha-Gal Positive Control | 1 vial | -20°C |

PLEASE NOTE: Assay Buffer 25 was previously labelled as Assay Buffer XXV and a-Gal Assay Buffer, and alpha-Gal Positive Control as a-Gal Positive Control, and Stop Solution V as a-Gal Stop Buffer. The composition has not changed.

## 5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom is preferred. 96-well clear plate can also be used.
- Multi-well spectrophotometer.
- Dounce Tissue Homogenizer.

# 6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

### 6.1 Assay Buffer 25:

Store at -20°C or 4°C. Bring to 37°C before use.

### 6.2 Stop Solution V:

Store at -20°C. Bring to 37°C before use.

### 6.3 a-Gal Substrate:

Light sensitive. Thaw at room temperature. Store at -20°C.

### 6.4 4-Methylumbelliferone Standard:

Light sensitive. Thaw at room temperature. Store at -20°C.

### 6.5 a-Gal Positive Control:

Reconstitute with 20 µL of Assay Buffer 25 and mix thoroughly. Store at -20 °C. Keep on ice while in use. Use within two months.

# 7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

**7.1** Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µL of 5 mM 4-MU to 490 µL Assay Buffer 25 in amber tube.

**7.2** Further dilute the 100 µM Standard solution 5-fold by adding 20 µL of 100 µM 4-MU to 80 µL Assay Buffer 25 to generate 20 µM 4-MU Standard.

**7.3** Add 0, 2, 4, 6, 8, and 10 µL of 20 µM 4-MU standard into a series of wells to generate 0, 40, 80, 120, 160 and 200 pmol of 4-MU Standard respectively. Adjust the volume to 60 µL/well with Assay Buffer 25.

| Standard # | 20 µM 4-MU Standard (µL) | Assay Buffer 25 (µL) | 4-MU standard/well |
|---|---|---|---|
| 1 | 10 | 50 | 200 pmol |
| 2 | 8 | 52 | 160 pmol |
| 3 | 6 | 54 | 120 pmol |
| 4 | 4 | 56 | 80 pmol |
| 5 | 2 | 58 | 40 pmol |
| 6 | 0 | 60 | 0 pmol |

**ΔNote:** Equilibrate the Assay Buffer 25 to 37 °C prior to the assay.

# 8. Sample Preparation

8.1 For tissue and cells: Homogenize tissue (10 mg) or pelleted cells (~5 x $10^5$) with 100 µL ice-cold Assay Buffer 25 and keep on ice for 10 minutes. Centrifuge samples at 12,000 x *g* at 4°C for 10 minutes and collect the supernatant. Dilute the supernatant 10-20 fold in Assay Buffer 25. Add 2-10 µL of diluted samples into a 96-well plate that will be designated as Sample(s).

8.2 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.

8.3 For Reagent Background Control: Add same volume of Assay Buffer 25 in parallel well(s).

8.4 For Positive Control: Dilute reconstituted a-Gal Positive Control 1:10 fold with Assay Buffer 25 prior to the assay and add 2-6 µl of diluted a-Gal Positive Control into desired wells(s).

8.5 Adjust the volume of Positive Control, Sample(s), and Reagent Background Control to 40 µL/well with Assay Buffer 25.

**ΔNote**:

- 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.
- Do not store unused diluted a-Gal Positive Control.
- We recommend saving an aliquot of sample homogenate to determine sample protein concentration using the Bradford reagent, BCA Assay or a comparable protein assay.

# 9. Assay Procedure

**9.1** Prepare sufficient volume of 10-fold dilution of a-Gal Substrate (i.e. Dilute 4 µL of a-Gal stock Substrate with 36 µL of Assay Buffer 25), vortex briefly.

**9.2** Add 20 µL of diluted a-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.

**9.3** Mix well and incubate at 37 °C for 2 hours, avoid light.

**9.4** After incubation, add 200 µL of Stop Solution V to each well containing Sample(s), Positive Control, Reagent Background Control and Standards. Mix well.

**Δ Note:** Equilibrate the Stop Solution V to 37 °C prior to the assay.

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

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

## 10. Data Analysis

10.1 Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve.

10.2 Subtract the Reagent Background Control reading from all Sample readings.

10.3 Apply sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (B, in pmol) and calculate the activity of a-Galactosidase activity in the sample as:

$$\text{Specific Sample a-GAL Activity (pmol/h/mg)} = B/(2 \times V \times P) \times D$$

To convert a-GAL Activity from unit of pmol/h/mg to µU/mg, multiply the activity obtained from the equation above by 0.0167 pmol/min.

Where: **B** is amount of 4-MU in the sample well from Standard Curve (pmol)

**2** is the reaction time (hour)

**V** is sample volume added into the reaction well (mL)

**P** is the protein concentration of the undiluted sample in mg-protein/mL

**D** is the sample dilution factor

1 pmol/h= 0.0167 pmol/min ≡ 0.0167 µU

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

## 11. Typical Data

Typical data provided **for demonstration purposes only**.

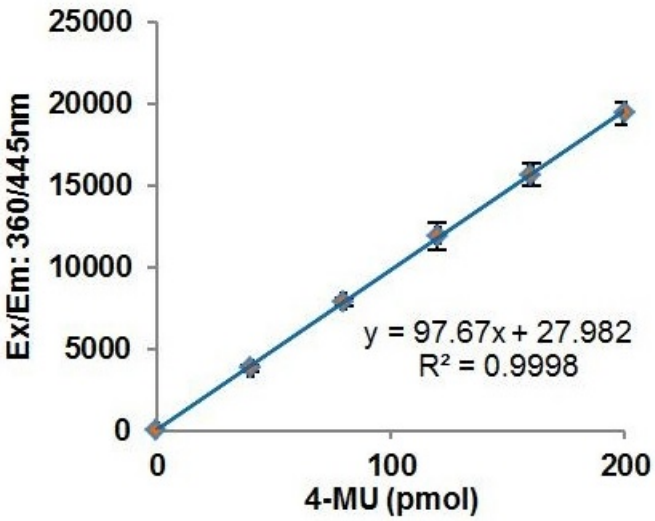


**Figure 1**. 4-Methylumbelliferon Standard Curve. Results are from multiple experiments.

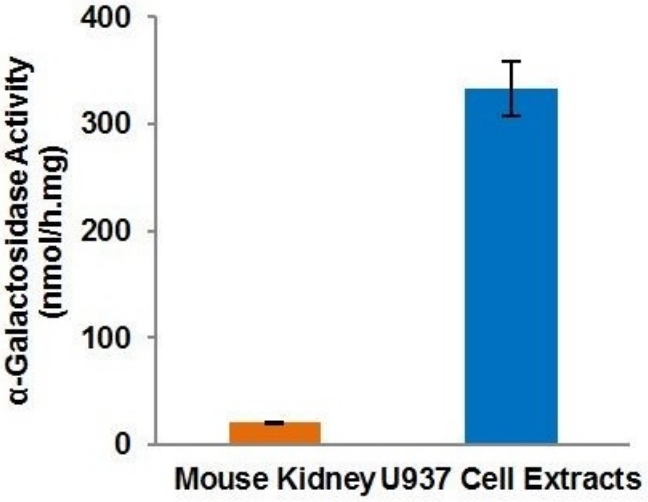


**Figure 2.** a-Galactosidase Acitvity in Mouse kidney tissue extracts (1 µg protein) and U937 cell lysate (0.2 µg protein).

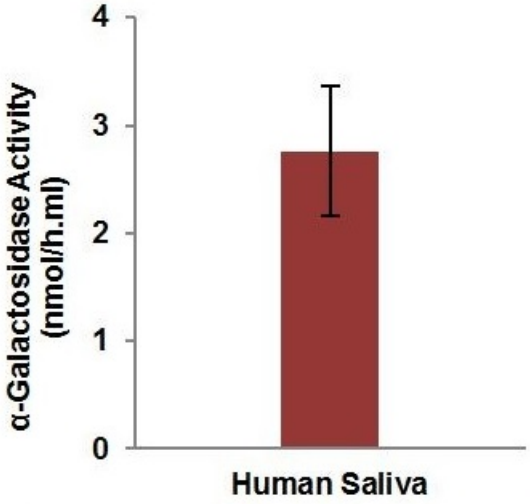


**Figure 3.** Measurement of a-Galactosidase Acitvity in undiluted human pooled saliva (5 µL). All assays were performed following kit protocol.

# 12. Notes

## Technical Support



### For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)