Version 4b, Last Updated: 18 March 2026

ab252887 Lysosomal alpha-Glucosidase Activity Assay Kit (Fluorometric)

For the measurement of Lysosomal alpha-Glucosidase (GAA)activity in tissue homogenates, cell lysates and other biological fluids.

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

Table of Contents

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1. Overview

Lysosomal alpha-Glucosidase Activity Assay Kit (Fluorometric) ab252887 provides a simple way to monitor GAA activity in a wide variety of biological samples.

In this kit, GAA cleaves a synthetic specific substrate releasing a fluorophore, which can be easily quantified (Ex/Em= 368/460 nm). The assay is specific and sensitive, it can detect as low as 0.05 µU of GAA activity.

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2. Protocol Summary

Prepare tissue, cell or other biological fluid samples.

Prepare standard curve.

Prepare GAA Substrate and add to all wells except standards.
Mix well and incubate at 37°C for 90 mins in the dark.

Add Stop Solution V to all wells.

Measure fluorescence intensity (Ex/Em=368/460 nm) at 37°C with end point setting using a fluorescence microtiter plate reader.

Calculate GAA activity.

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3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 25	25 mL	-20°C	4°C or -20°C
Stop Solution V	25 mL	-20°C	-20°C
GAA Substrate	280 µL	-20°C	-20°C
4-Methylumbelliferone Standard	35 µL	-20°C	-20°C
GAA Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 25 was previously labelled as Assay Buffer XXV and GAA Assay Buffer, and Stop Solution V as GAA Stop Buffer, and GAA Substrate as GAA Substrate (in DMSO). The composition has not changed.

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4. Materials Required, Not Supplied

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

- Multi-well spectrophotometer (ELISA reader)
- 96-well clear flat bottom plate
- Dounce tissue homogenizer
- 10 kDa cut-off spin column

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5. 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.

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6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Assay Buffer 25

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

6.2 Stop Solution V

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

6.3 GAA Substrate

Ready to use as supplied. Light sensitive, thaw at room temperature.

6.4 4-Methylumbelliferone Standard

Ready to use as supplied. Light sensitive, thaw at room temperature.

6.5 GAA Positive Control

Reconstitute with 100 µL Assay Buffer 25. Pipet up and down to mix thoroughly. Aliquot and Store at -20°C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.

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7. Sample Preparation

For tissue and cells:

7.1 Homogenize tissue (~10-20 mg) or pelleted cells (~1 x 10^6) with 500 µL ice-cold Assay Buffer 25 and keep on ice for 10 mins.

7.2 Centrifuge samples at 12,000 x *g* at 4°C for 10 mins and collect the supernatant.

7.3 For concentrated samples: prepare samples 1:10 fold or higher dilution in Assay Buffer 25.

7.4 Add 2-10 µL of prepared samples into well(s) of a 96-well clear flat bottom plate with clear flat wells as Sample.

For serum:

7.5 Endogenous molecules may interfere with the results. Therefore, remove these interferences by filtering serum samples through a 10 kDa cut-off spin column (10K x *g* at 4°C, 10 mins), discard the filtrate. Adjust the ultraconcentrate to the original volume using Assay Buffer 25 and repeat this procedure 3-5 times.

For every experiment:

7.6 Add 40 µL of Assay Buffer 25 in separate well(s) as a Background Control.

7.7 For Positive Control: Prepare a 10-fold dilution of reconstituted GAA Positive Control (i.e. Dilute 10 µL of reconstituted GAA Positive Control with 90 µL Assay Buffer 25).

7.8 Add 5 µL of Diluted GAA Positive Control into desired well(s). Adjust the volume of Positive Control and Sample wells to 40 µL/well with Assay Buffer 25.

Δ Note: We suggest using 3-5 different amounts of the samples per well to ensure the readings are within the standard curve range and the changes of rates are within the linear range of the curve.

Δ Note: Do not store unused diluted GAA Positive Control.

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8. Standard Curve Preparation

∆**Note** Equilibrate the Assay Buffer 25 to 37°C before adding to the wells.

8.1 Prepare a 100 µM 4-Methylumbelliferone Standard (4-MU) by adding 2 µL of 5 mM 4-MU to 98 µL Assay Buffer 25; further dilute the 100 µM 4-MU Standard solution 5-fold to 20 µM 4-MU Standard: i.e. add 20 µL of 100 µM 4-MU to 80 µL Assay Buffer 25.

8.2 Add 0, 2, 4, 6, 8, 10 µL of 20 µM (20 pmol/µL) 4-MU standard into a series of wells to generate 0, 40, 80, 120, 160, 200 pmol of 4-MU/well respectively.

8.3 Adjust the volume to 60 µL/well with Assay Buffer 25.

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

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9. Assay Procedure

9.1 **Substrate Hydrolysis:** Prepare 8-fold dilution of GAA Substrate (i.e. Dilute 10 µL of GAA stock Substrate with 70 µL of Assay Buffer 25), vortex briefly. Prepare sufficient amount of substrate (20 µL per well) for all wells except the wells used for standards. Using a multichannel pipette add 20 µL of prepared GAA Substrate to each well(s) containing the test Samples, Positive Control and Background Control. The total volume in each well (i.e. Sample, Positive Control and Reagent Background Control) should be 60 µL. Mix well and incubate at 37°C for 90 mins protected from light.

Δ Note: Prepare working concentration of substrate right before use and discard unused diluted GAA Substrate.
Δ Note: Ensure the GAA Substrate and Assay buffer are at room temperature to improve solubility of the substrate during dilution. The final dilution can be incubated at 37°C for 5 min if necessary.

9.2 **GAA Assay:** After 90 mins incubation, add 100 µL of Stop Solution V to each well containing Sample(s), Positive Control, Background Control and Standards. Mix well. The final total volume in each well should be 160 µL.

Δ Note: Equilibrate Stop Solution V to 37°C prior to the assay.
Δ Note: Standards can be prepared at the end of the incubation time and measured in end-point mode.

9.3 **Measurement:** Measure fluorescence intensity (Ex/Em=368/460 nm) at 37°C with end point setting using a fluorescence microtiter plate reader.

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9.4 **Calculation:** Subtract 0 Standard reading from all Standard(s) readings. Plot the 4-MU Standard Curve; subtract the Background Control reading from Sample readings. Apply sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmoles of product formed (B, in pmoles) and calculate the activity of Lysosomal a-Glucosidase activity in the sample as:

Sample GAA Activity = B/ (90 x V x P) x D = pmol/min•mg (μU/mg)

Where:

B is 4-MU amount from Standard Curve (pmoles)
90 is Reaction time (min)
V is Sample volume added into the reaction well (ml)
P is Initial Sample Concentration in mg-protein/ml (mgP/ml)
D is Sample Dilution Factor

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

* GAA specific activity can be expressed as nmol/ml.h (16.7 μU/ml) or nmol/mg.h (16.7 μU/mg).

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10. Typical Data

Data provided **for demonstration purposes only**.

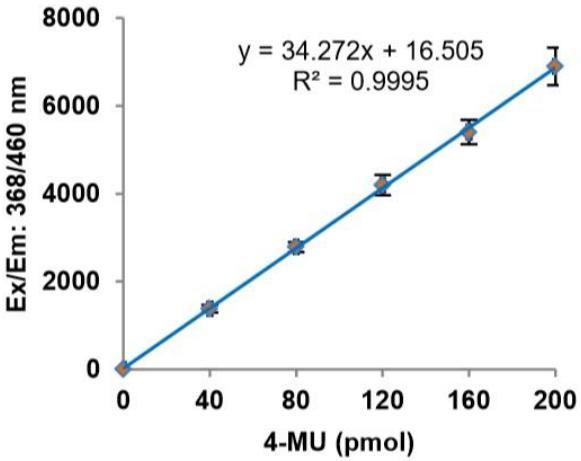

Figure 1. 4-Methylumbelliferone (4-MU) Standard Curve, results from multiple experiments.

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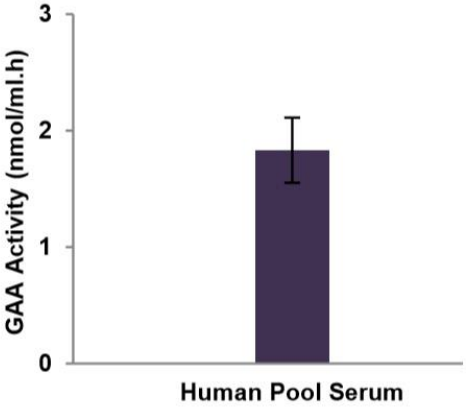

Figure 2. Measurement of GAA activity in human pool serum (5 µL, ultraconcentrate).

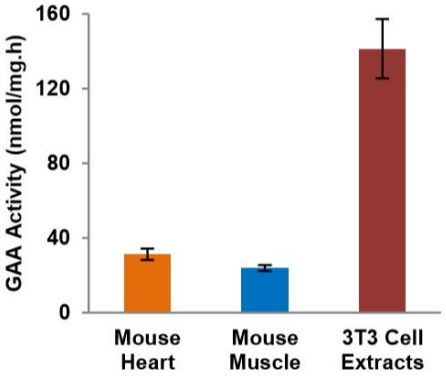

Figure 3. Measurement of GAA activity in mouse heart extracts (3 µg protein), mouse muscle extracts (2 µg protein) and 3T3 cell lysates (0.5 µg protein).

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11. Notes

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