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Lysosomal α-Glucosidase (GAA) Activity Assay Kit (Fluorometric)

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(Catalog # K187-100; 100 assays; Store at -20°C)

I. Introduction:

Lysosomal α-Glucosidase (GAA, Acid α-Glucosidase, Acid Maltase; EC 3.2.1.3) is an exo-1,4 and exo-1,6-α-Glucosidase, which is essential for the degradation of glycogen to glucose in lysosomes. Deficiency in GAA activity results in the accumulation of glycogen within the lysosome, leading to Glycogen storage disease type II [also termed acid maltase deficiency (AMD) or Pompe disease]. Pompe disease is an inherited disorder of glycogen metabolism with broad spectrum of clinical phenotypes. Cardiac and skeletal muscles are the major target tissues and GAA activity in dry blood spots has been used to screen, and diagnose Pompe Disease. BioVision's Lysosomal α-Glucosidase (GAA) Activity Assay Kit 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, sensitive- it can detect as low as 0.05 µU of GAA activity.

$$\text{GAA Substrate} \xrightarrow{\text{Lysosomal α-Glucosidase}} \text{Cleaved Substrate + Fluorescent Product (Ex/Em= 368/460 nm)}$$

II. Applications:

- Measurement of Lysosomal α-Glucosidase activity in various tissues/cells.

III. Sample Type:

- Tissue Homogenates: Muscle, Heart, etc.
- Cell Lysates: 3T3 Cell Lysates, etc.
- Biological fluids: Serum, etc.

IV. Kit Contents:

Components	K187-100	Cap Code	Part Number
GAA Assay Buffer	25 ml	NM	K187-100-1
GAA Stop Buffer	25 ml	WM	K187-100-2
GAA Substrate (in DMSO)	280 µl	Blue	K187-100-3
4-Methylumbelliferone Standard (5 mM)	35 µl	Yellow	K187-100-4
GAA Positive Control	1 vial	Green	K187-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- 96-well clear plate with flat bottom
- Dounce Tissue Homogenizer (Cat. #1998)

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.

- **GAA Assay Buffer and Stop Buffer:** Store at either 4 ºC or -20 ºC. Bring to 37 ºC before use.
- **GAA Substrate & 4-Methylumbelliferone Standard:** Light sensitive, thaw at room temperature. Store at -20 °C.
- **GAA Positive Control:** Reconstitute with 100 µl GAA Assay Buffer. Pipet up and down to mix thoroughly. Aliquot and Store at -20 ºC. Avoid freeze/thaw. Use within two months. Keep on ice while in use.

VII. GAA Activity Assay Protocol:

1. **Sample Preparation: For Tissue and cells:** Homogenize tissue (~10-20 mg) or pelleted cells (~1 x 10^6) with 500 µl ice-cold GAA 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. *For concentrated samples: prepare samples 1:10 fold or higher dilution in GAA Assay Buffer*. Add 2-10 µl of prepared samples into well(s) in a 96-well clear plate as Sample. **For Serum:** *Endogenous molecules may interfere with the results.* Therefore, remove these interferences by filtering Serum Samples through a 10 kDa cut-off spin column (BioVision #1997; 10K x g at 4 ºC, 10 min), discard the filtrate. Adjust the ultraconcentrate to the original volume using GAA Assay Buffer and repeat this procedure 3-5 times. Add 5-10 µl of ultraconcentrate into well(s) in a 96-well clear plate as Sample. ***For every experiment, add 40µl of GAA Assay Buffer in separate well(s) as Background Control***. **For Positive Control:** Prepare a 4-fold dilution of reconstituted GAA positive Control (i.e. Dilute 10 µl of reconstituted GAA Positive Control with 30 µl GAA Assay Buffer). Add 5-15 µl Diluted GAA Positive Control into desired well(s). Adjust the volume of Positive Control and Sample wells to **40 µl/well** with GAA Assay Buffer.

   Note:

   a. 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.

   b. Do not store unused diluted GAA Positive Control.

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone Standard (4-MU) by adding 2 µl of 5 mM 4-MU to 98 µl GAA Assay Buffer; 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 GAA Assay Buffer. 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. Adjust the volume to **60 µl/well** with GAA Assay Buffer.

Note: Equilibrate the GAA Assay Buffer to 37 ºC before adding to the wells.

3. **Substrate Hydrolysis:** Prepare 8-fold dilution of GAA Substrate (i.e. Dilute 10 µl of GAA stock Substrate with 70 µl of GAA Assay Buffer), vortex briefly. Prepare sufficient amount of substrate (20 µl per well) for all wells except the wells used for standards. Using multichannel pipette and aAdd **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 min., protect from light**.

   Note: Prepare working concentration of substrate right before use & discard unused diluted GAA Substrate.

4. **GAA Assay:** After 90-min incubation, add 100 µl of GAA Stop Buffer 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.**

   Note:

   a. Equilibrate GAA 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.

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

6. **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 pmol of product formed (**B**, in pmol) and calculate the activity of Lysosomal α-Glucosidase activity in the sample as:

   Specific Sample Lysosomal α-Glucosidase Activity = B/ (90 x V x P) x D = pmol/min.mg (µU/mg)

   Where: **B** is 4-MU amount from Standard Curve (pmol)
   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 α-Glucosidase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone (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).

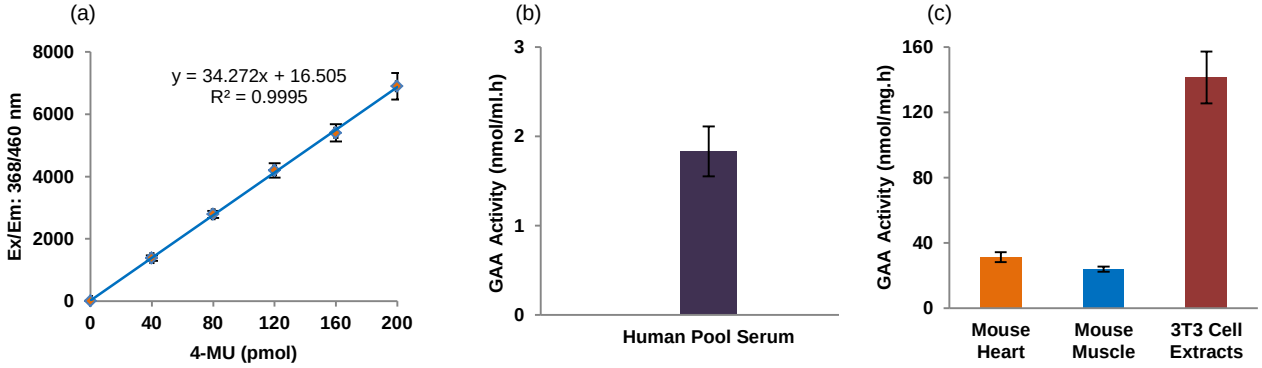

Figure: (a) 4-Methylumbelliferone (4-MU) Standard Curve, results from multiple experiments. (b) Measurement of GAA Activity in Human Pool Serum (5 µl, ultraconcentrate). (c) Measurement of GAA Activity in Mouse Heart Extracts (3 µg protein), Mouse Muscle Extracts (2 µg protein) and 3T3 Cell Lysates (0.5 µg protein). All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

α-Glucosidase Activity Colorimetric Assay Kit (K690)
α-Glucosidase Inhibitor Screening Kit (K938)
Starch Colorimetric/Fluorometric Assay Kit (K647)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Glucose Colorimetric Assay Kit II (K686)
Glucose-6-phosphate Dehydrogenase Assay Kit (K757)
Glucose Uptake Colorimetric Assay Kit (K676)
Glycogen Colorimetric/Fluorometric Assay Kit (K646)
Hexokinase Colorimetric Assay Kit (K789)
Maltose Colorimetric/Fluorometric Assay Kit (K628)
Total Carbohydrate Assay Kit (K645)
Amylase Activity Colorimetric Assay Kit (K711)
Dounce Tissue Homogenizer (1998)
Glucose Colorimetric/Fluorometric Assay Kit (K666)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose Dehydrogenase Activity Assay Kit (K786)
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glucose Uptake Fluorometric Assay Kit (K666)
Glycogen Colorimetric Assay Kit II (K648)
PicoProbe™ Glucokinase Activity Assay Kit (K969)
Maltose & Glucose Colorimetric/Fluorometric Assay Kit (K618)
PicoProbeTM Glucose-6-Phosphate Assay Kit (K687)

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