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Alpha-Mannosidase Activity Assay Kit (Fluorometric)

rev 03/20

(Catalog # K2041-100; 100 assays; Store at -20°C)

I. Introduction:

Alpha-Mannosidase (α-Man; EC 3.2.1.24) is a glycoside hydrolase involved in the cleavage of the alpha-mannose. α-Man can be applied in studies that determine the effects of mannose on specific molecules, such as recombinant proteins that are used for vaccine development. Lysosomal α-Man is essential for catabolizing the Asn-linked glycans of glycoproteins and is actively involved in maintaining the cellular homeostasis. α-Man deficiency results in Alpha-Mannosidosis, an acquired lysosomal storage disorder in which α-Man fails to break down mannose-containing oligosaccharides. These oligosaccharides, therefore, accumulate in lysosomes thereby leading to cellular malfunction and apoptosis. **BioVision's Alpha-Mannosidase Activity Assay Kit** provides a facile, rapid way to monitor total α-Man activity in various biological samples. In this kit, α-Man cleaves a synthetic specific substrate and releases a fluorophore, which can be easily quantified (Ex/Em = 360/445 nm). The substrate is specific to α-Man and can differentiate from β-mannosidase. The assay is simple, sensitive and can detect as low as 1.0 µU of alpha mannosidase activity.

α-Mannosidase Substrate $\xrightarrow{\text{α-Mannosidase}}$ Cleaved Substrate + Fluorescent Product (Ex/Em = 360/445 nm)

II. Application:

- Measurement of alpha-mannosidase activity in various samples

III. Sample Types:

- Biological fluids: Human plasma, etc.
- Cell Lysates: 3T3 cells, etc.
- Tissue homogenates: Rat liver, etc.

IV. Kit Contents:

Components	K2041-100	Cap Code	Part Number
α-Man Assay Buffer	25 ml	NM	K2041-100-1
α-Man Stop Buffer	25 ml	WM	K2041-100-2
α-Man Substrate	100 µl	Blue	K2041-100-3
4-Methylumbelliferone Standard	35 µl	Yellow	K2041-100-4
α-Man Positive Control	1 vial	Green	K2041-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **α-Man Assay Buffer and α-Man Stop Buffer:** Store at 4°C or -20°C. Bring to room temperature (RT) before use.
- **α-Man Substrate:** Light sensitive, protect from light. Thaw at RT. Store at -20°C.
- **4-Methylumbelliferone Standard:** Light sensitive, protect from light. Thaw at RT. Store at -20°C.
- **α-Man Positive Control:** Reconstitute with 100 µl α-Man Assay Buffer and mix thoroughly. Divide into aliquots & store at -20°C. Keep on ice while in use. Avoid multiple free-thaw cycles. Use within two months.

VII. α-Man Activity Assay Protocol:

1. **Sample Preparation: For tissues and cells:** Homogenize tissues (10 mg) or pelleted cells (~5 x 10^5) with 100 µl ice-cold α-Man Assay Buffer and keep on ice for 10-15 min. Centrifuge samples at 12,000 x *g* and 4°C for 10 min and collect the supernatant. Dilute the supernatant 5-10 fold in α-Man Assay Buffer. Add 2-10 µl of diluted samples into a 96-well clear plate designated as Sample(s).

   For biological fluids: Undiluted fluids can be added directly to the wells. Add 2-10 µl of samples into well(s) of a 96-well clear plate designated as Samples. **For Reagent Background Control**: Add same volume of α-Man Assay Buffer in parallel well(s).

   For Positive Control: Dilute the reconstituted α-Man Positive Control 10 fold with α-Man Assay Buffer prior to the assay. Add 2-6 µl of the diluted α-Man Positive Control into desired wells(s).

   Adjust the volume of Positive Control, Sample(s), and Reagent Background Control wells to **50 µl/well** with α-Man Assay Buffer.

   Notes:

   a. We suggest using several dilutions of the Samples to ensure the readings are within the Standard Curve range.

   b. Do not re-use the diluted α-Man Positive Control.

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 4-MU stock solution to 490 µl α-Man Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of 100 µM 4-MU standard into a series of wells to generate 0, 200, 400, 600, 800, 1000 pmol/well of 4-MU Standard respectively. Adjust the volume to **60 µl/well** with α-Man Assay Buffer.

3. **Substrate Hydrolysis:** Prepare sufficient volume of 10-fold dilution of the α-Man Substrate (i.e. dilute 10 µl of α-Man Substrate with 90 µl of α-Man Assay Buffer), vortex briefly. Add 10 µl of the diluted α-Man Substrate to each well containing the Sample(s), Positive Control and Reagent Background Control. The total volume of each well (i.e. Samples, Positive Control and Reagent Background Control) should be **60 µl**. **Mix well and incubate at 37°C for 15 min, protected from light**. After incubation, add 200 µl of α-Man Stop Buffer to all the wells including Sample(s), Positive Control, Reagent Background Control, and Standards. Mix well.

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

4. **Measurement:** Measure fluorescence intensity (Ex/Em = 360/445 nm) at 37°C in end-point mode.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve. Subtract the Reagent Background Control reading from all Sample readings to get the corrected Sample readings (ΔRFU). Apply the corrected Sample readins (ΔRFU) to 4-MU Standard Curve to obtain the corresponding pmol of product formed **(B, in pmol)** and calculate the activity of α-Mannosidase in the Sample as:

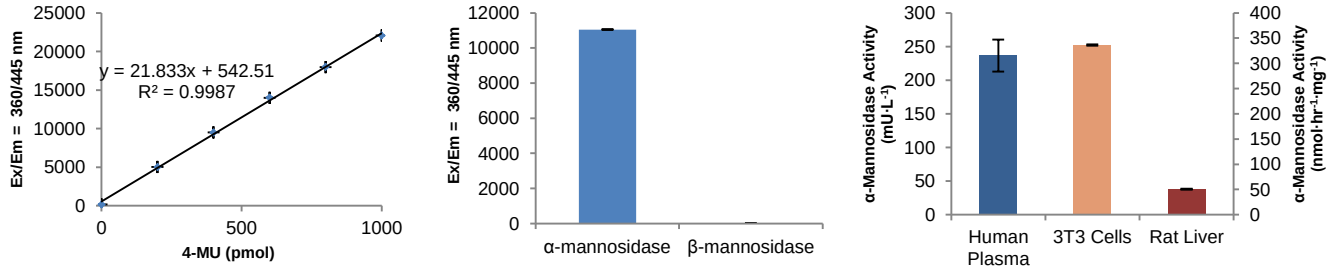
Sample α-Mannosidase Activity = B / (0.25 x V x P) x D (pmol/hr/mg ≡ 0.0167 µU/mg)

Where: **B** = 4-MU amount in Sample well from the Standard Curve (pmol)
0.25 = Reaction time (hr)
V = Sample volume added into the reaction well (ml)
P = Initial Sample concentration (mg/ml)
D = Sample dilution factor (D= 1 for undiluted samples)

1 pmol/hr = 0.0167 pmol/min = 0.0167 µU

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

a) **b)** **c)**



Figures: (a). 4-Methylumbelliferone (4-MU) Standard Curve. (b). Measurement of purified α-Mannosidase (0.6 ng) and β-Mannosidase (43 ng) activities using our proprietary substrate. The kit can efficiently distinguish α-Mannosidase activity from β-Mannosidase. (c). α-Mannosidase activity in human plasma (10 µl), 3T3 cells (2 µg protein), and rat liver (10 µg protein). All assays were performed following the kit protocol.

VIII. Related Products:

Alpha Galactosidase (α-Gal) Activity Assay Kit (Fluorometric) (K407)
Beta Galactosidase (β-Gal) Activity Assay Kit (Fluorometric) (K821)
Glucosylceramidase Activity Assay Kit (Fluorometric) (K2003)
Alkaline Sphingomyelinase Activity Assay Kit (Colorimetric) (K987)
Acid Sphingomyelinase Assay Kit II (Colorimetric) (K192)
α-L-Fucosidase (FUCA1) Assay Kit (Colorimetric) (K224)
α-Glucosidase Activity Colorimetric Assay Kit (K690)
Dounce Tissue Homogenizer (1998)

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