ab282918 – Beta-Mannosidase Activity Assay Kit (Fluorometric)

For the measurement of beta-mannosidase activity in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282918

Storage and Stability

Entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
β-Man Assay Buffer	25 mL	-20°C
β-Man Stop Buffer	25 mL	-20°C
β-Man Substrate	100 µl	-20°C
4-Methylumbelliferone Standard	35 µl	-20°C
β-Man Positive Control	1 vial	-20°C

Materials Required, Not Supplied

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

- Multi-well spectrophotometer (ELISA reader)
- 96-well clear plate with flat bottom
- Dounce Tissue Homogenize

Reagent Preparation

Before using the kit, spin the tubes prior to opening.
β-Man Assay Buffer and β-Man Stop Buffer: Store at 4°C or -20°C. Bring to room temperature (RT) before use.
β-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.
β-Man Positive Control: Reconstitute with 100 µl β-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.

Assay Protocol

Sample Preparation

For tissues and cells: Homogenize tissues (10 mg) or pelleted cells (~5 x 105) with 100 µl ice-cold β-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 β-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 2-10 µl of β-Man Assay Buffer in parallel well(s).
For Positive Control: Dilute the reconstituted β-Man Positive Control 10 fold with β-Man Assay Buffer prior to the assay. Add 2-6 µl of the diluted β-Man Positive Control into desired wells(s).
Adjust the volume of Positive Control, Sample(s), and Reagent Background Control wells to 40 µl/well with β-Man Assay Buffer.
Notes:

- We suggest using several dilutions of the Samples to ensure the readings are within the Standard Curve range.
- Do not re-use the diluted β-Man Positive Control.

Standard Curve Preparation

1. Prepare a 100 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 4-MU stock solution to 490 µl β-Man Assay Buffer.
2. 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.
3. Adjust the volume to 60 µl/well with β-Man Assay Buffer

Substrate Hydrolysis

1. Prepare sufficient volume of 20-fold dilution of the β-Man Substrate immediately before the assay (i.e. dilute 10 µl of β-Man Substrate with 190 µl of β-Man Assay Buffer), vortex briefly.
2. Add 20 µl of the diluted β-Man Substrate to each well containing the Sample(s), Positive Control and Reagent Background Control.
3. The total volume of each well including Samples, Positive Control and Reagent Background Control should be 60 µl. Mix well and incubate at 37°C for 20 min, protected from light.
4. After incubation, add 200 µl of β-Man Stop Buffer to all the wells including Sample(s), Positive Control, Reagent Background Control, and Standards. Mix well.

Notes:
Prepare the diluted β-Man Substrate solution immediately before the assay and do not re-use the diluted solution.
Standards can be prepared at the end of the incubation time and measured in end-point mode

Measurement

Measure fluorescence intensity of all wells at 37°C in end-point mode at Ex/Em = 360/445 nm.

Calculation:

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

Sample β-Mannosidase Activity = B x 3/ (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)
3 = Inverse of 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 beta-mannosidase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone per min, at pH 4.5 at 37°C.

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)