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# β-Xylosidase Activity Assay Kit (Fluorometric)

8/16

**(Catalog # K981-100; 100 assays; Store kit at -20°C)**

## I. Introduction:

β-Xylosidase (EC 3.2.1.37) is a hydrolytic enzyme responsible for the breakdown of hemicellulose, a primary component of the plant cell wall. Specifically, β-Xylosidases remove the terminal β-xylose residue from the non-reducing terminus of a polysaccharide chain. As xylan is the principal variety of hemicellulose present in the plant cell wall, the xylosidase activity is the final step in the enzymatic generation of the five carbon reducing sugar, xylose. Individual reducing sugars can be converted to ethanol, which serves as a building block for biofuels. Xylose can also be converted into other value-added chemicals such as Xylitol and Xylonic Acid. BioVision's β-Xylosidase Activity Assay Kit provides a quick, reliable, and sensitive fluorometric method for the determination of β-Xylosidase activity. A fluorogenic substrate is incubated with the sample, and in the presence of β-Xylosidase activity, the substrate is cleaved, releasing a fluorophore (Ex Em 330/450 nm). The increase in fluorescence is a direct measure of the β-Xylosidases activity in the sample. When used according to protocol, activities as low as one µUnit (1 pmol/min) in environmental microbial samples, such as compost, can be detected.

## II. Applications:

- Determination of β-Xylosidase activity in samples
- Characterization of purified β-Xylosidase

## III. Sample Type:

- Purified recombinant protein
- Environmental Samples (compost, e.g.)

## IV. Kit Contents:

| Components | K981-100 | Cap Code | Part Number |
|---|---|---|---|
| β-Xylosidase Assay Buffer | 25 ml | NM | K981-100-1 |
| β-Xylosidase Substrate (in DMSO) | 250 µl | Red | K981-100-2 |
| β-Xylosidase Positive Control | 1 vial | Orange | K981-100-3 |
| 4-Methylumbelliferone Standard (5 mM) | 35 µl | Yellow | K981-100-4 |

## V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- Black 96-well plate

## VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the experiment.

- **β-Xylosidase Assay Buffer**: Ready to use. Warm to room temperature before use. Store at -20°C.
- **β-Xylosidase Substrate and 4-Methylumbelliferone Standard (4-MU):** Ready to use. Warm to RT before use. Store at -20°C. Use within six months.
- **β-Xylosidase Positive Control**: Reconstitute with 55 µl of β-Xylosidase Assay Buffer to prepare the stock solution. Store at 4 °C. Use within two months.

## VII. β-Xylosidase Activity Assay Protocol:

1. **Sample Preparation:** For compost and dirt samples: add 1:1 w/v water to sample (e.g. 10 ml to 10 g compost). *Incubate 6-48 hours*; pellet insoluble material with centrifugation at 5,000 X g for 5 min. Collect the supernatant. Add 5-50 µl supernatant into a well of an opaque black 96-well plate. For the positive control reaction, use 5 µl of the reconstituted Positive Control. Adjust the volume of each reaction to 90 µl with β-Xylosidase Assay Buffer).

   **Notes:**

   **a.** For unknown samples, we suggest trying several dilutions to ensure results are in the linear range of the Standard Curve.

   **b.** For samples having high background, prepare parallel sample background controls (see step 3).

2. **4-Methylumbelliferone Standard Curve Preparation:** Prepare a 200 µM 4-Methylumbelliferone (4-MU) by adding 10 µl of 5 mM 4-MU to 240 µl β-Xylosidase Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl of 200 µM 4-MU standard into a series of wells to generate 0, 0.4, 0.8, 1.2, 1.6, and 2.0 nmole (2000 pmole) of 4-MU/well respectively. Adjust the volume by adding β-Xylosidase Assay Buffer to 100 µl.

3. **Substrate Mix:** Dilute β-Xylosidase Substrate (in DMSO)to working concentration by adding 3 volumes of Assay Buffer to 1 volume of β-Xylosidase Substrate for the Substrate Working Stock, e.g. for 10 assays, combine 25 µl β-Xylosidase Substrate with 75 µl Assay Buffer. If lower numbers of wells are needed, 2.5 µl of β-Xylosidase substrate can be diluted with 7.5 µl β-Xylosidase Assay Buffer for each well. To initiate reaction, add 10 µl Substrate Mix to each sample and positive control well. *Measure Fluorescence at 37°C in kinetic mode.*

**Notes:**

**a.** Diluted substrate can be stored at -20°C for 2 months.

**b.** No Substrate (Background) Control: For lysates and environmental samples, prepare a parallel sample background reaction by omitting the substrate mix and instead adding 10 µl β-Xylosidase Assay Buffer.

**c.** Do not add substrate to 4-MU standard wells.

**4. Measurement:** Immediately after addition of substrate, measure the fluorescence in a kinetic mode at 37°C for 60 min. (Ex/Em = 330/450 nm).

**Note:**

**a.** Incubation time depends on the β-Xylosidase activity in samples. We recommend measuring the fluorescence in kinetic mode, and choosing two time points ($t_1$ & $t_2$) in the linear range to calculate the β-Xylosidase activity of the samples.

**b.** The 4-MU Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

**5. Calculation:** Subtract 0 Standard reading from all readings. Plot the 4-MU Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the β-Xylosidase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the 4-MU Standard Curve to get B pmol of 4-MU generated during the reaction time ($\Delta t = t_2 - t_1$).

**Sample β-Xylosidase Activity = B/(Δt X V) x D = pmol/min/ml = µU/ml**

Where: **Δt** = reaction time (min.)
**B** = 4-MU amount from Standard Curve (pmol)
**V** = sample volume added into the reaction well (ml)
**D** = Dilution Factor

**Unit Definition:** 1 Unit is defined as the amount of β-Xylosidase that can cleave 1 µmol of substrate/min under the assay condition at 37°C.

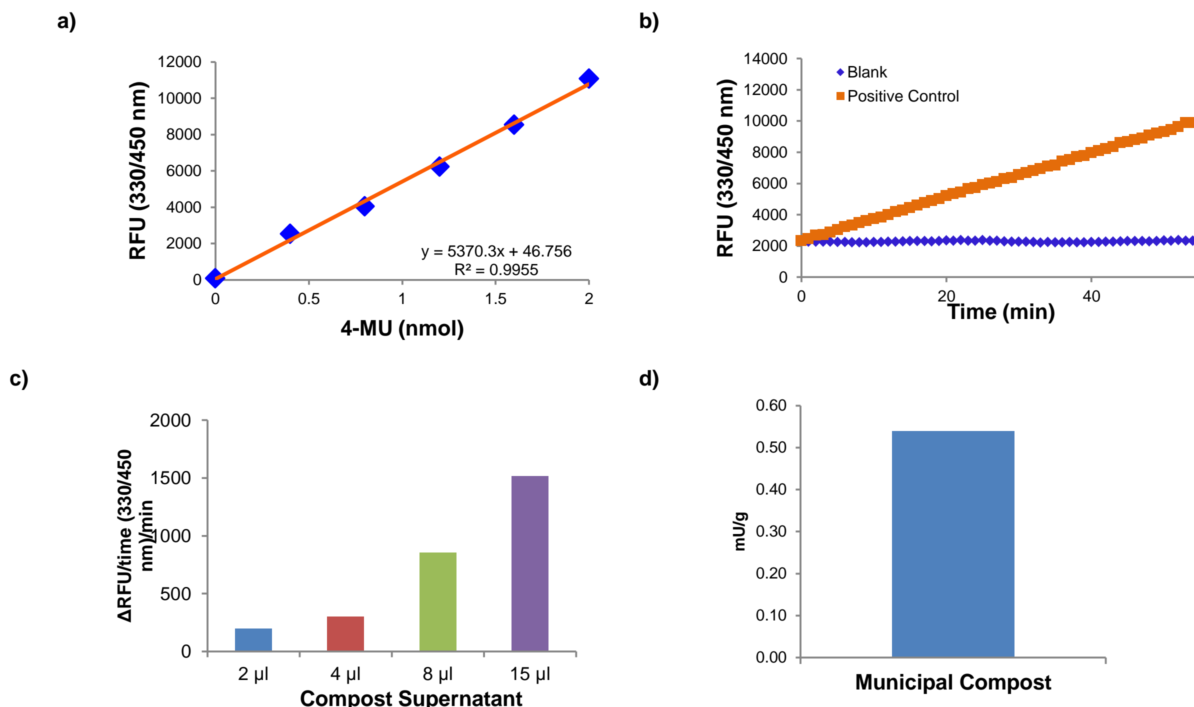


**Figure:** (a) 4-Methylumbelliferone Standard Curve; (b) Time course using positive control β-Xylosidase as described above in *Sample Preparation*; (c) and (d) Example of determination of β-Xylosidase activity in compost sample. Compost (10 g) was incubated for 48 hr. at room temperature in tap water (10 ml), at which point the resulting broth was clarified by centrifugation. Rates were determined by incubation of given volumes of resultant broth with β-Xylosidase Substrate as described for 2 hours.

## VIII. RELATED PRODUCTS:

β-Glucuronidase Activity Assay Kit (K245)

Amylase Activity Colorimetric Assay Kit (K711)

***FOR RESEARCH USE ONLY! Not to be used on humans.***