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Cellulase Activity Assay Kit (Fluorometric)

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(Catalog # K2080-100; 100 assays; Store at -20 °C)

I. Introduction:

Cellulases are enzymes that catalyze the decomposition of cellulose molecule into monosaccharides such as beta-glucose, or shorter polysaccharides and oligosaccharides. They are produced by fungi, bacteria, and protozoans. Cellulase is a complex of three different enzymes including β-1,4-endoglucanase, cellobiohydrolase, and β-glucosidase. Cellulose is a major component of the plant cell wall and most mammals have a very limited ability to digest cellulose by themselves. So cellulose breakdown by fungal cellulases make a major constituent of plants available for consumption. Most fungal cellulases have a two-domain structure, a catalytic domain and a cellulose binding domain. Cellulase is used for commercial food processing in coffee. Additionally, cellulases are widely used in textile industry, laundry detergents, pulp and paper industry, pharmaceutical applications etc. **BioVision's Cellulase Activity Assay Kit** provides a facile, rapid way to measure cellulase activity in various biological samples. In this kit, cellulase cleaves a synthetic substrate and releases the fluorophore, which can be easily quantified at Ex/Em = 365/450 nm. The substrate is specific for cellulase and can differentiate its activity from that of 1,3-β-D-glucanase. The assay is simple, sensitive and can detect cellulase activity lower than 500 nU in samples.

Cellulase Substrate —(**Cellulase** / **Cellulase Developer**)→ Cleaved Substrate + Fluorescent Product (Ex/Em = 365/450 nm)

II. Application:

- Measurement of cellulase in various samples.

III. Sample Types:

- Grains: Barley seeds, etc.
- Fungus: Mushrooms, etc.

IV. Kit Contents:

Components	K2080-100	Cap Code	Part Number
Cellulase Assay Buffer	25 ml	NM	K2080-100-1
Cellulase Stop Buffer	25 ml	WM	K2080-100-2
Cellulase Developer	1 vial	Green	K2080-100-3
Cellulase Substrate	600 µl	Blue	K2080-100-4
4-Methylumbelliferone Standard	35 µl	Yellow	K2080-100-5
Cellulase Positive Control	1 vial	Violet	K2080-100-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

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

- **Cellulase Assay Buffer and Cellulase Stop Buffer:** Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.
- **Cellulase Developer:** Reconstitute the vial in 220 µl dH_2O. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **Cellulase Substrate:** Ready to use. Thaw at RT. Store at -20 °C.
- **4-Methylumbelliferone Standard:** Store at -20 °C, protected from light. Thaw at RT for the assay.
- **Cellulase Positive Control:** Reconstitute the vial in 55 µl Cellulase Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.

VII. Cellulase Activity Assay Protocol:

1. **Sample Preparation: for Grains or Fungus:** Weigh out 10-50 mg of the sample, cut into small pieces, if needed. Transfer sample into an eppendorf tube and homogenize in 100-300 µl ice-cold Cellulase Assay Buffer using a Dounce Tissue Homogenizer (BioVision Cat# 1998). Keep on ice for 10-15 min. Centrifuge at 12,000 x *g* and 4 °C for 15 min and collect the supernatant. Add 2-10 µl of the supernatant samples into a 96-well clear plate designated as Sample(s).
   For Positive Control: Dilute Cellulase Positive Control to 5 fold with Cellulase Assay Buffer prior to the assay. Add 2-6 µl of diluted Cellulase Positive Control into a parallel well(s) labeled as Positive Control.
   For Reagent Background Control: Add 30 µl Cellulase Assay Buffer to a well labeled as Reagent Background Control.
   Adjust the volume of Sample(s) and Positive Control wells to 30 µl/well with Cellulase Assay Buffer.
   Notes:
   a. For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range.
   b. Do not re-use the diluted Cellulase Positive Control.

2. **Standard Curve Preparation:** Prepare 500 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 4-MU stock solution to 90 µl Cellulase Assay Buffer. Further dilute the 500 µM 4-MU Standard solution at 1:10 dilution to obtain 50 µM 4-MU Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 50 µM 4-MU Standard solution into a series of wells to generate 0, 100, 200, 300, 400, 500 pmole/well of 4-MU Standard respectively. Adjust the volume to 60 µl/well with Cellulase Assay Buffer.

   Note: Standards can be prepared during the incubation step of Substrate Hydrolysis.

3. **Substrate Hydrolysis:** Mix enough reagents for the number of assays to be performed. Prepare 30 µl of Reaction Mix as indicated in the table below:

	Reaction Mix
Cellulase Assay Buffer	22 µl
Cellulase Developer	2 µl
Cellulase Substrate	6 µl

   Add 30 µl of Reaction Mix to each well containing Sample, Positive Control, and Reagent Background Control and mix well. The total volume of each well including Sample, Positive Control, and Reagent Background Control is 60 µl. **Incubate the assay plate at 40 °C for 15 min, protected from light.** After incubation, add 200 µl of Cellulase Stop Buffer to all the wells including Sample(s), Positive Control, Reagent Background Control, and Standards. Mix well.

   Note: Prepare Reaction Mix immediately before adding to the wells.

4. **Measurement:** Measure the fluorescence intensity of all wells at 40 °C in end-point mode at Ex/Em = 365/450 nm.

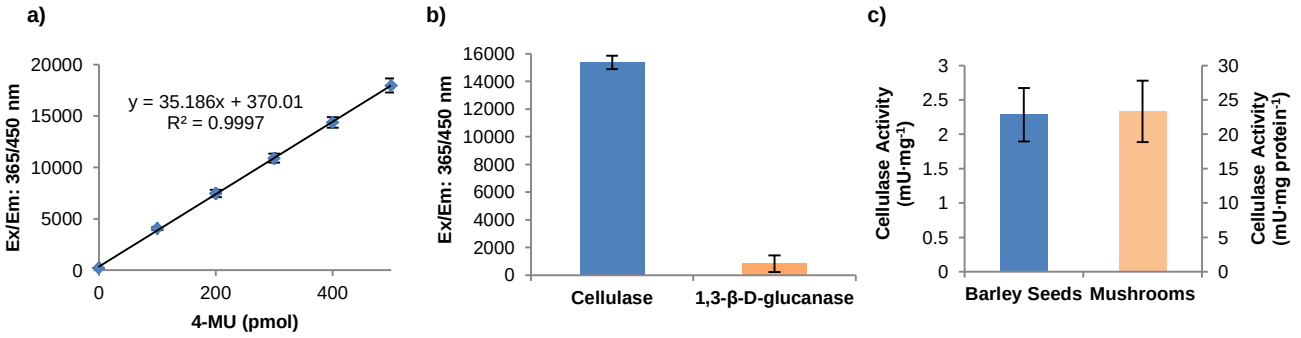
5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve. Subtract the Reagent Background Control reading from all Sample readings to get the corrected Sample readings (ΔRFU). Apply the corrected Sample readings (ΔRFU) to the 4-MU Standard Curve to obtain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

   Sample Cellulase Activity = B x 4 x D / (V x P) (pmol/hr/mg ≡ 0.0167 µU/mg)

   Where: **B** = 4-MU amount in the Sample well from the Standard Curve (pmol)
   4 = Inverse of reaction time (hr)
   V = Sample volume added into the reaction well (ml)
   P = Initial Sample concentration (mg (protein)/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 Cellulase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone per min, at pH 4.5 at 40 °C.



Figures. a. 4-MU Standard Curve, results from multiple experiments. **b.** Measurement of purified cellulase (2.5 ng) and 1,3-β-D-glucanase (2.5 ng) activities using BioVision's proprietary substrate. The kit can efficiently distinguish the cellulase activity from 1,3-β-D-glucanase. **c).** Cellulase activity in barley seeds (5 mg) and mushrooms (40 µg protein). All assays were performed following kit protocol.

VIII. Related Products:

Beta-Glucan Assay Kit (Fluorometric) (K2068)
Alpha-Mannosidase Activity Assay Kit (Fluorometric) (K2041)
Beta-Mannosidase Activity Assay Kit (Fluorometric) (K2045)
Glucosylceramidase Activity Assay Kit (Fluorometric) (K2003)
Dounce Tissue Homogenizer (1998)

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