AB308326– Cellulase Activity Assay Kit (Fluorometric)

Measurement of cellulase in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XXV/Cellulase Assay Buffer	25 ml	4 °C or -20 °C
Stop Solution V/Cellulase Stop Buffer	25 ml	4 °C or -20 °C
Cellulase Developer	1 vial	-20 °C
Cellulase Substrate (Avoid light)	600 µl	-20 °C
4-Methylumbelliferone Standard (Avoid light)	35 µl	---
Cellulase 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:

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

Reagent Preparation

Assay Buffer XXV/Cellulase Assay Buffer and Stop Solution V/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 dH2O. 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 Assay Buffer XXV/Cellulase Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.

Cellulase Activity Assay Protocol:

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 Assay Buffer XXV/Cellulase Assay Buffer using a Dounce Tissue Homogenizer. 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 Assay Buffer XXV/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 Assay Buffer XXV/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 Assay Buffer XXV/Cellulase Assay Buffer.

Δ Note:

1. *For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range.*
2. *Do not re-use the diluted Cellulase Positive Control.*

Standard Curve Preparation:

Prepare 500 µM 4-Methylumbelliferone (4-MU) Standard by adding 10 µl of 4-MU stock solution to 90 µl Assay Buffer XXV/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 Assay Buffer XXV/Cellulase Assay Buffer.

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

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
Assay Buffer XXV/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 Stop Solution V/Cellulase Stop Buffer to all the wells including Sample(s), Positive Control, Reagent Background Control, and Standards. Mix well.

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

Measurement

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

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.

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)