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ab189817 Cellulase Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of cellulase activity in plant tissues.

[View kit datasheet: www.abcam.com/ab189817](http://www.abcam.com/ab189817)
(use www.abcam.cn/ab189817 for China, or www.abcam.co.jp/ab189817 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Cellulase Activity Assay Kit (Fluorometric) (ab189817) provides a simple method to measure cellulase activity in plant tissues, as well as purified cellulase extracted from plants, bacteria or fungi. The assay uses a long-wavelength fluorescent substrate, resorufin cellobioside. Upon cleavage of the substrate by cellulases present in the sample, the fluorescent compound resorufin is released and fluorescence can be easily detected at Ex/Em = 550/595 nm (peak Ex/Em = 571/585 nm) in a fluorescent microplate reader. The amount of fluorescence will correlate with cellulase activity.

This assay can detect as low as 50 μ U/ml of cellulase activity.

Cellulases (EC 3.2.1.4) are a family of enzymes that include β -Glucosidases, endoglucanases, and exoglucanases. These enzymes cleave the β -1,4-D-glycosidic bonds that link the glucose units comprising cellulose. In addition to being produced by plants, cellulase activity is found in many fungi and bacteria, including some plant pathogens. Most animal cells are not known to produce cellulase; cellulolytic activity is often carried out in animals by symbionts. However, recent evidence does suggest cellulase production in some animals, such as insects and arthropods. The study of cellulase activity has many applications in plant molecular biology, agriculture, and manufacturing. Cellulase is also becoming important in the development of alternative fuel sources, as glucose obtained from cellulose hydrolysis is easily fermented into ethanol.

2. Protocol Summary

Standard curve preparation



Sample preparation



Add Substrate reagent



Measure fluorescence (Ex/Em = 571/585 nm)
[range Ex/Em = 550/590 nm] in kinetic mode
for 10-120 minutes at 25°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Refer to list of materials supplied for storage conditions of individual components and store in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
DMSO	5 mL	-20°C
Reaction Buffer	30 mL	-20°C
Reference Standard (resorufin, 5 mM in DMSO)	250 µL	-20°C
Stop Buffer	10 mL	-20°C
Substrate Reagent (resorufin-cellobioside, 5 mM in DMSO)	1 mL	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 571/585 nm.

Δ Note: These are the peak wavelengths of the substrate fluorescence. However, the Stokes shift of these may be too narrow for some instruments, as indicated by very high reading across the entire plate, especially in the blank wells. In that is the case, adjust settings to Ex/Em = 550/595 nm.

- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably black
- Anhydrous DMSO (reagent grade)
- Pestle and mortar
- Liquid nitrogen: to freeze/ground tissues

8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 DMSO:

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Store at -20°C.

9.2 Reaction Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 Reference Standard (5 mM resorufin in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Wrap vial in foil to protect from light.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time standard is needed.

Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze/thaw.

9.4 Stop Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.5 Substrate Reagent (5 mM resorufin-cellobioside in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Wrap vial in foil to protect from light.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time substrate is needed.

Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze/thaw.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 0.5 mM standard by diluting 10 μL of the provided 5 mM resorufin standing in 90 μL of DMSO.

10.2 Using the 0.5 mM working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to dilute	Volume standard in well (μL)	Reaction Buffer (μL)	End conc resorufin in well
1	0.5 mM stock	50	450	50 μM
2	Std #1	250	250	25 μM
3	Std #2	250	250	12.5 μM
4	Std #3	200	200	6.25 μM
8	Blank (none)	0	300	0 nM

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 μL).

Δ Note: self-quenching begins to occur at a concentration of 50 μM Resorufin reference standards should be kept below this concentration.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Plant tissue lysates:

- 11.1.1 Harvest tissue, seeds or flowering buds necessary for each assay (initial recommendation: 10 mg).
- 11.1.2 Freeze tissue in liquid nitrogen.
- 11.1.3 Ground to a fine powder using pestle and mortar.
- 11.1.4 Resuspend powder in 200 μL Reaction buffer by pipetting up and down a few times.
- 11.1.5 Centrifuge sample for 10 minutes at 4°C at 10,000 $\times g$ using a cold microcentrifuge to remove any insoluble material.
- 11.1.6 Collect supernatant and transfer to a new tube.
- 11.1.7 Keep on ice.

11.2 Bacteria cell lysates:

- 11.2.1 Harvest cells necessary for the assay.
- 11.2.2 Resuspend cells in 1X volume of Reaction buffer.
- 11.2.3 Lyse bacterial cells: this can be done by French press, ultrasonication or freeze:thaw cycles.
- 11.2.4 Centrifuge sample for 10 minutes at 4°C at 10,000 $\times g$ using a cold microcentrifuge to remove any insoluble material.
- 11.2.5 Collect supernatant and transfer to a new tube.
- 11.2.6 Keep on ice.

11.3 Purified enzyme:

No sample preparation is necessary. Dilute as required in Reaction Buffer to fit standard curve.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- In the absence of a fluorometric plate reader, the assay may also be performed by monitoring the increase in absorbance at OD 570nm in a colorimetric plate reader. However, sensitivity of the assay will be decreased compared to fluorometric assay. In this case, the range of the standard curve might need to be adjusted.

Δ Note: Non-specific hydrolysis of the Substrate reagent may occur over time if assays are performed over a long period of time (several hours). We recommend setting up Background Controls to correct for potential non-specific hydrolysis.

12.1 Plate loading:

- Standard wells = 100 μ L standard dilutions.
- Sample wells = 1 - 50 μ L samples (adjust volume to 50 μ L/well with Reaction Buffer).
- Background control wells = 50 μ L Reaction Buffer.

12.2 Reaction:

12.2.1 Prepare a 0.5 mM substrate reagent working solution by diluting 5 mM Substrate reagent 1:10 in reaction buffer (ie. 100 μ L 5 mM Substrate Reagent + 900 μ L Reaction Buffer).

Δ Note: Substrate should be diluted immediately prior starting the assay to prevent non-specific hydrolysis of substrate

12.2.2 Add 50 μ L of 0.5 mM substrate into each sample well and background control wells. Do not add Substrate to the standard wells.

12.3 Measurement:

12.3.1 Measure immediately fluorescence at Ex/Em = 550/595 nm (peak Ex/Em = 571/585 nm) in a microplate reader in kinetic mode, every 3 minutes, for 10-120 minutes at 25°C.

Δ Note: Incubation time depends on the cellulase activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the cellulase activity of the samples. Read Standard Curve along with the samples.

End-point reading measurement:

If you are performing the assay in endpoint reading mode, follow this procedure:

- Incubate plate at 25°C (room temperature) for the desired reaction time
- Add 30 μ L Stop Buffer to each sample well and background control wells.
- Measure fluorescence at Ex/Em = 550/595 nm (peak Ex/Em = 571/585 nm).

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
 - Use only the linear rate for calculation.
- 13.1 Subtract the mean fluorescence value of the blank (Reaction buffer) from all the standard and sample readings. This is the corrected fluorescence
- 13.2 Standard curve calculation:
- 13.2.1 Average the duplicate reading for each standard.
- 13.2.2 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).
- 13.3 Measurement of Cellulase activity in the sample:
- 13.3.1 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding fluorescence values at those points (RFU1 and RFU2).
- 13.3.2 Calculate Δ RFU for sample as follows:

$$\Delta\text{RFU} = \text{RFU2} - \text{RFU1}.$$

- 13.3.3 If sample background control reading is significant, subtract sample background control reading from sample reading.
- 13.3.4 Apply the Δ RFU to Resorufin Standard Curve to get B pmol of resorufin generated during the reaction time.
- 13.3.5 HK activity in the test samples is calculated as:

$$\text{Cellulase Activity} = \left(\frac{B}{\Delta T * V} \right) * D$$

Where:

B = amount of resorufin in sample well calculated from Standard Curve (μmol)

ΔT = linear phase reaction time $T_2 - T_1$ (minutes).

V = original sample volume added into the reaction well (μL).

D = sample dilution factor if sample is diluted to fit within the standard curve range.

14. Typical Data

Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

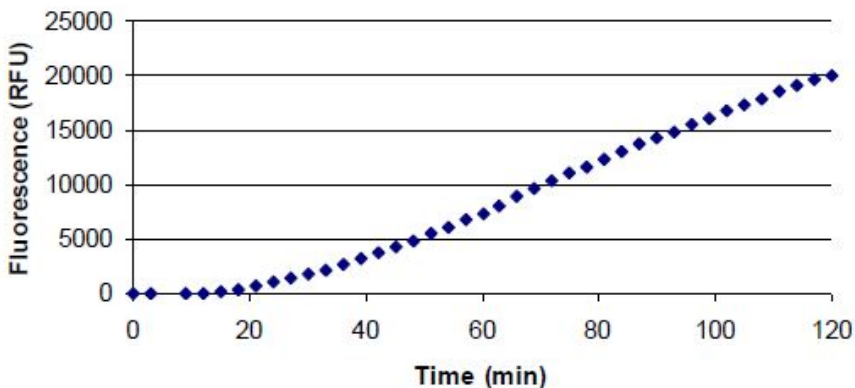


Figure 1. Cellulase activity in lysates from *Arabidopsis thaliana* (CS-20). Flowering buds (0.09 g) from two mature *Arabidopsis* plants (strain CS-20) were removed from plant and lysates were prepared following sample preparation protocol. Fluorescence readings at Ex/Em = 550/595 nm were taken at 3-minute intervals for 120 minutes. Fluorescence values of background control wells (50 μ L reaction buffer + 50 μ L Substrate reagent) were subtracted at each time point.

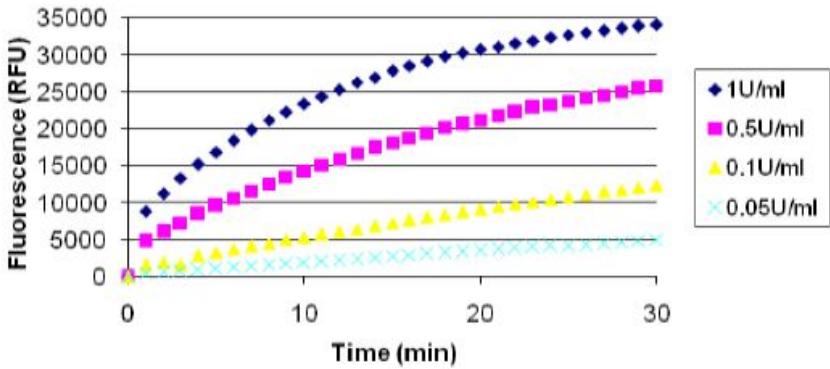


Figure 2. Cellulase activity from purified cellulase from the fungus *Trichoderma reesei*. The purified enzyme was diluted in Reaction buffer (0.05-1 U/mL). Fluorescence readings at Ex/Em = 550/595 nm were taken at 3-minute intervals for 30 minutes. Fluorescence values of background control wells (50 μ L reaction buffer + 50 μ L Substrate reagent) were subtracted at each time point.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Thaw reagents and aliquot; get equipment ready.
- Prepare resorufin standard dilution [0.625-50 μM /well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (100 μL), samples (50 μL) and background sample control wells (50 μL).
- Prepare 0.5 mM Substrate reagent working solution by (1:10 dilution in Reaction Buffer)
- Add 50 μL 0.5 mM Substrate reagent solution to sample wells and background control.
- Measure plate at Ex/Em = 550/595 nm in kinetic mode.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μ L) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQs

Q. Do I need to set up a reference standard curve?

A. The reference standard solution included in the kit is for:

- a) calibrating your reader, such as gain setting, filter setting etc;
- b) determining the linear range of resorufin, which is the product of the cellulase assay.

All assays should generate product which is in the linear detection range by fluorometer or fluorescence plate reader.

Q. Our sample contains cellulases that are present in quite low amounts. Will this kit work for us?

A. Fluorescence is probably the most sensitive assay available. It is about 2-3 orders of magnitude more sensitive than other chromogenic techniques. We were able to measure about 50 $\mu\text{U}/\text{mL}$ without any difficulties.

18. Notes

Technical Support

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