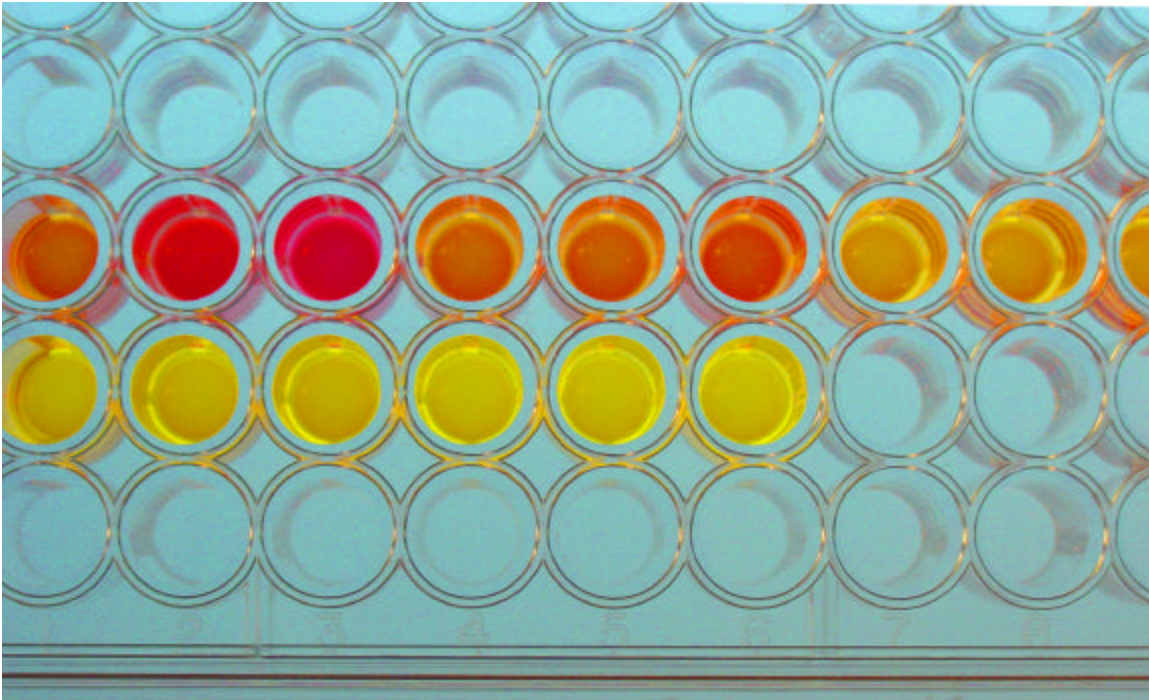




Product Information Sheet (M1245-007)
MarkerGene™ Fluorescent Cellulase Assay Kit
(Product M1245)





MarkerGene™ Fluorescent Cellulase Assay Kit (Product M1245)

NOTE: The following information is given as a viable methodology for use of MarkerGene™ Fluorescent Cellulase Assay Kit. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

Cellulases are a family 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. Activity of most cellulases can be monitored using our long wavelength fluorescent substrate, Resorufin Cellobioside (Product M1238), contained in the kit. Upon cleavage, the fluorescent compound, Resorufin (Product M0202) is released and activity measurements are easily obtained in a microtiter plate based assay format. The kit contains enough substrate for 200 assays and control experiments (100 μ L reaction volume) and also contains reference standards and a detailed protocol for use. See the references below for more information and applications.

II. MATERIALS

A.) Substrate Reagent: 5mM Resorufin Cellobioside in Dimethyl sulfoxide (DMSO). Dilute to 0.5mM for use in the assay protocol below.

B.) Reference Standard: 5mM Resorufin in Dimethyl sulfoxide (DMSO). Dilute to desired concentration using supplied DMSO and reaction buffer.

C.) Reaction Buffer: This buffer contains 100mM Sodium Acetate, pH 6.0.

D.) Stop Buffer: This buffer contains 165mM Tris Base, pH 9.3

E.) Dimethyl Sulfoxide



F.) Storage and Handling: The substrate reagent and reference standard included in this kit should be kept cold when not in use and stored at -20°C. Protect solutions of the substrate reagent and reference standard from light.

III. ENZYME ASSAY

It is recommended that a calibration curve be generated using concentrations of the Reference Standard in a range from 0 to the highest concentration of substrate reagent used for all assays. Several concentrations of the Reference Standard (M1245-003) can be made by dilution in DMSO (M1245-005).

In addition, purified enzyme assays should be performed using several enzyme concentrations in the approximate or estimated range of the enzyme concentration expected for the unknown sample. To normalize data, each enzyme reaction should be subtracted from a blank (no enzyme) sample.

- 1.) It is recommended that samples to be assayed are diluted at least 1:1 in reaction buffer (Product M1245-001). Enzyme can be extracted from solid material (such as plant tissue) by freezing in liquid nitrogen and grinding in a mortar, then suspending the resulting powder in reaction buffer (see Figure 1 below). Presence of solid material in sample may affect fluorescence readings. Solid materials should be removed by centrifugation prior to measurement if possible. Keep samples in an ice bath until needed.
- 2.) Purified enzyme concentrations should be prepared fresh by diluting enzyme in reaction buffer (Product M1245-001). Keep samples in an ice bath until needed.
- 3.) To a 96-well microtiter plate, add samples and purified enzyme solutions to wells in triplicate (50 μ L/well). Also include in triplicate wells for blanks and reference standards (50 μ L reaction buffer/well).
- 4.) Prepare reference standard solutions by diluting 5mM reference standard solution (Product No. M1245-003) to 10X desired concentration in DMSO (Product No. M1245-005). Dilute 10X solution 1:10 in reaction buffer (Product No. M1245-001) to reach final concentration. Mix solution and let stand for 5 minutes prior to use. To wells to be used as reference standards, add prepared reference standard solution. (100 μ L) (see note (2) below). If a standard curve is desired to produce kinetic data, reference standard solutions should be prepared at several concentrations



and added to wells. Concentration of standard may vary depending on enzyme concentration or sensitivity of reader.

- 5.) Prepare a 0.5mM substrate reagent solution by diluting 5mM substrate reagent (100 μ L) (Product No. M1245-002) in reaction buffer (900 μ L) (Product No. M1245-001). Prepare this solution just prior to performing the assay (see note (1) below). Add 0.5mM substrate reagent (50 μ L/well) to all wells, except those containing reference standard.
- 6.) Read fluorescence (Ex/Em = 571/585 nm) in a microtiter plate reader, using appropriate filters. Use the wells containing reference standard to optimize reading conditions. If kinetic data is desired, readings may be taken beginning immediately after addition of the substrate reagent, and at several defined time points thereafter. If only an endpoint reading is needed, plate should be incubated at room temperature for the desired time, and fluorescence recorded after the addition of stop buffer (30 μ L/well) (Product M1245-004).
- 7.) Average the readings of duplicate samples. Subtract fluorescence of blanks from that of each sample in order to normalize data. (See Note (1) below).
- 8.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. time (log-log).
- 9.) Using the calibration curve generated in step 8, determine the activity (concentration) of the enzyme in the sample, and extrapolate this data to determine the original enzyme concentration.

Note (1.) 0.5mM substrate reagent should be prepared just prior to performing the assay to prevent nonspecific hydrolysis of substrate. A small amount of nonspecific hydrolysis may occur over time if assays are performed over a long period (several hours). Potential hydrolysis may necessitate the need for blank wells as recommended in the assay protocol.

Note (2.) In addition to generating kinetic data, use of reference standard wells may be useful in optimizing reading conditions of the microtiter plate reader.

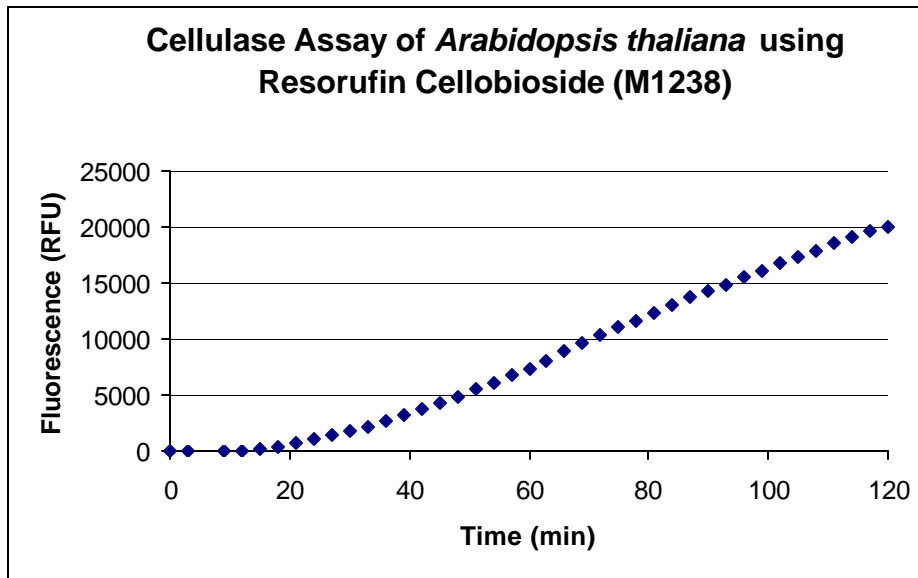


Figure 1: Flowering buds from two mature *Arabidopsis thaliana* plants (strain CS-20) were removed (0.09g tissue) and ground to a fine powder in liquid nitrogen. Powder was suspended in reaction buffer (200 μ L) (Product M1245-001) and centrifuged (13000 rpm) for 10 minutes. Supernatant was collected and added in triplicate (50 μ L) to wells on a 96-well microtiter plate (clear, flat bottom). A 0.5mM substrate reagent was prepared by diluting 5mM Resorufin Cellobioside (in DMSO) (Product M1245-002) 1:10 in reaction buffer and was added to wells (50 μ L/well). Fluorescence was recorded using a Perkin-Elmer HTS 7000 BioAssay Reader, with 550nm excitation filter and 595nm emission filter. Fluorescence readings were taken at 3-minute intervals for 120 minutes. Fluorescence values of blank (50 μ L substrate reagent added to 50 μ L reaction buffer) were subtracted at each time point.

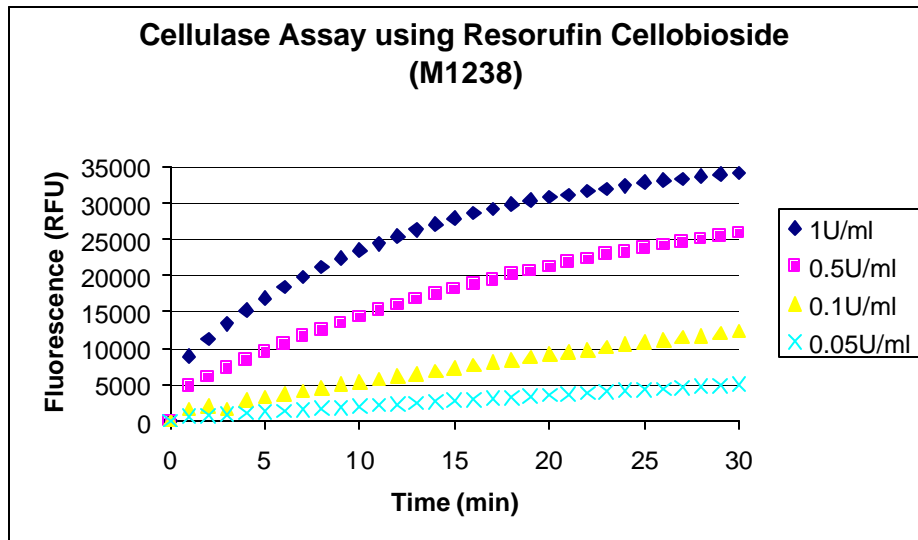


Figure 2: Several dilutions of purified cellulase from *Trichoderma reesei* (FLUKA 22173) were prepared in reaction buffer (Product M1245-001). Each preparation was added in triplicate (50 μ L) to wells on a 96-well microtiter plate (clear, flat bottom). A 0.5mM substrate reagent was prepared by diluting 5mM Resorufin Cellobioside (in DMSO) (Product M1245-001) 1:10 in reaction buffer and was added to wells (50 μ L/well). Fluorescence was recorded using a Perkin-Elmer HTS 7000 BioAssay Reader, with 550nm excitation filter and 595nm emission filter. Fluorescence readings were taken at 1-minute intervals for 30 minutes. Fluorescence values of blank (50 μ L substrate reagent added to 50 μ L reaction buffer) were subtracted at each time point.

M1245 Kit Contents

Description	Quantity	Part No.	Storage
Reagents			
Reaction Buffer	1 x 30 mL vial	1245-001	C,R
Substrate Reagent (5mM Resorufin Cellobioside in DMSO)	1 x 1 mL vial	1245-002	F,R,L
Reference Standard (5mM Resorufin in DMSO)	1x 250 μ L vial	1245-003	F,R,L
Stop Buffer	1 x 10 mL vial	1245-004	C
Dimethyl Sulfoxide (DMSO)	1 x 5 mL vial	1245-005	R,RT,FL
Documentation			
MSDS Sheets	3	1245-006	N/A
Product Information Sheet	1	1245-007	N/A

Notes: F=store at or below -20^o C; RT=store at room temperature; C=store cold (4^o C); L=light sensitive; FL=flammable; R=read protocol instructions carefully prior to use.

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