

# Megazyme

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## **endo-CELLULASE** ASSAY PROCEDURE (CellG5 METHOD)

12/19

K-CellG5-4V

(120/240 Manual Assays per Kit) or  
(480 Auto-Analyser Assays per Kit)

K-CellG5-2V

(60/120 Manual Assays per Kit) or  
(240 Auto-Analyser Assays per Kit)

(EP 2740800 A1)



## INTRODUCTION:

Cellulase (*endo*-1,4- $\beta$ -glucanase) plays a key role in the hydrolysis of cellulosic biomass to fermentable sugars. This enzyme also finds widespread industrial applications in the modification of cellulosic materials and in the degradation of mixed linkage 1,3;1,4- $\beta$ -glucans.

Numerous methods are available for the measurement of cellulase, including those based on increase in reducing sugar levels on hydrolysis of CM-cellulose (e.g. **P-CMC4M**) or 1,3:1,4- $\beta$ -glucan<sup>1</sup> (**P-BGBM**). *endo*-Cellulase can be specifically assayed using viscometric methods with barley  $\beta$ -glucan (e.g. CMC-7M) or by employing soluble or insoluble (crosslinked) dyed cellulose (**S-ACMCL**, **I-AZCEL**, **T-CCZ**) or mixed-linkage  $\beta$ -glucan (**S-ABGI00**, **I-AZBGL**, **T-BGZ**). In general, assays based on the use of dyed polysaccharides are standardised against a reducing sugar method that employs either CM-cellulose or  $\beta$ -glucan as substrate.<sup>2</sup> A defined substrate would be more desirable as any batch to batch variability would be eliminated. In addition, a soluble colourimetric substrate would be particularly useful for application in automated analysis assay systems where the use of dyed polysaccharide substrates is more difficult due to the filtration step required in these assay procedures.

The **CellG5** cellulase test reagent represents a significant improvement on the **CellG3** reagent that was introduced by Megazyme in 2012.<sup>3</sup> **CellG5** employs high purity thermostable  $\beta$ -glucosidase and blocked 4-nitrophenyl- $\beta$ -D-cellopentaoside (BPNPG5). The assay principle is outlined in Scheme 1, Appendix A (page 9). On hydrolysis of BPNPG5 by cellulase, the 4-nitrophenyl- $\beta$ -D-cellooligosaccharide fragment is immediately hydrolysed to D-glucose and free 4-nitrophenol (*p*NP) by the  $\beta$ -glucosidase present in the substrate mixture. Thus, the rate of release of *p*NP relates directly to the rate of hydrolysis of BPNPG5 by cellulase. The reaction is stopped and the phenolate colour is developed by addition of Tris buffer solution (pH 10). The linearity of the assay with different concentrations of *Trichoderma longibrachiatum* cellulase (**E-CELTR**) is shown in Figure 1 (page 10). Standard curves relating enzyme activity on CM-cellulose (**P-CMC4M**) to increase in absorbance at 400 nm on hydrolysis of BPNPG5 by *Trichoderma longibrachiatum* (**E-CELTR**), *Aspergillus niger* (**E-CELAN**), *Bacillus amyloliquefaciens* (**E-CELBA**) and *Thermotoga maritima* (**E-CELTM**) cellulases are outlined in Figure 2, Appendix C (page 10). This shows that relative hydrolytic activity on CellG5 versus CM-cellulose is very similar for each of the cellulases assayed. The assay can be used at temperatures up to 60°C and in the pH range of 4.5 to 8.0. Note that for the assay of cellulase enzymes with activity at high pH values, it is necessary to terminate the reaction with tri-sodium phosphate (pH 11.0) in place of Tris buffer solution.

## ACCURACY:

Standard errors of less than 3% are readily achieved (see Table 1, page 11).

## SPECIFICITY:

The assay is specific for cellulase (*endo*-1,4- $\beta$ -D-glucanase). The substrate is not hydrolysed by  $\beta$ -glucosidase, cellobiohydrolase or any other enzymes tested.

## SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

## KITS:

Two kit sizes are available from Megazyme:

**K-CellG5-4V** is suitable for performing 120/240 assays in manual format or 480 assays in auto-analyser format.

**K-CellG5-2V** is suitable for performing 60/120 assays in manual format or 240 assays in auto-analyser format.

### Bottle 1: (x2) or (x4)

Each vial contains 4,6-*O*-(3-Ketobutylidene)-4-nitrophenyl- $\beta$ -D-cellopentaoside (BPNPG5) in 10% DMSO/H<sub>2</sub>O (3 mL) plus sodium azide (0.02% w/v). Stable for > 4 years below -10°C.

### Bottle 2:

Thermostable  $\beta$ -glucosidase (0.50 mL, 600 U/mL) in 50% w/v ammonium sulphate solution plus sodium azide (0.02% w/v). Stable for > 4 years at 4°C.

### Bottle 3:

*Trichoderma* cellulase standard solution (5 mL, ~ 3 U/mL; actual value stated on the vial label) in 50% aqueous glycerol plus sodium azide (0.02% w/v). Stable for > 4 years; store sealed below -10°C.

## PREPARATION OF REAGENT SOLUTIONS:

- 1 & 2.** The CellG5 reagent solution is prepared by transferring 100  $\mu$ L of the suspension in bottle 2 (swirl bottle 2 to mix contents before use) to bottle 1 and mix well. The CellG5 reagent solution should only be prepared prior to use. Stable for ~ 4 weeks at 4°C or stable for ~ 5 days at room temperature.
- 3.** With a positive displacement pipette, dispense 0.5 mL of the contents of bottle 3 to 9.5 mL of **Buffer B** and mix well. Once diluted, the standard is stable for ~ 4 days at 4°C or ~ 6 months below -10°C.

## **BUFFERS:**

### **(A) Concentrated Acetate Buffer**

(Sodium acetate buffer, 1 M, pH 4.5)

Add 60.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH of this solution to 4.5 by the addition of 5 M (20 g/100 mL) NaOH solution. Adjust the volume to 1 L.

Stable for > 2 years at room temperature.

### **(B) Acetate Extraction/Dilution Buffer**

(Sodium acetate buffer, 100 mM, pH 4.5 containing 1 mg/mL BSA and 0.02% sodium azide)

Add 100 mL of concentrated acetate buffer (A) to 850 mL of distilled water. Adjust the pH to 4.5 by dropwise addition of 2 M HCl or 2 M NaOH and adjust the volume to 1 L. Add 1 g of BSA and dissolve. Add 0.2 g of sodium azide and dissolve. Stable for > 1 year at 4°C.

### **(C) Concentrated Phosphate Buffer**

(Sodium phosphate buffer, 0.5 M, pH 6.0)

Add 156 g of sodium dihydrogen orthophosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) to 1.5 L of distilled water. Adjust the pH to 6.0 with 4 M NaOH and adjust the volume to 2 L.

Stable for > 1 year at 4°C.

### **(D) Phosphate Extraction/Dilution Buffer**

(Sodium phosphate buffer, 100 mM, pH 6.0 containing 0.02% sodium azide)

Add 200 mL of concentrated phosphate buffer (C) to 750 mL of distilled water. Adjust the pH to 6.0 with 1 M HCl or 1 M NaOH and adjust the volume to 1 L. Add 0.2 g of sodium azide and dissolve.

Stable for > 1 year at 4°C.

## **NOTE:**

1. Do not add the sodium azide to the buffer until it has been adjusted to pH 4.5 or pH 6.0. Adding sodium azide to strongly acidic solutions can result in the release of a poisonous gas.
2. If diluted buffer is prepared without adding sodium azide as a preservative, then it should be stored on ice and used within a week. Alternatively, this can be stabilised against microbial contamination by storing the buffer in a well-sealed Duran<sup>®</sup> bottle and adding 1 drop of toluene.
3. The addition of BSA to the acetate extraction/dilution buffer is necessary to increase the stability of the diluted *Trichoderma* enzyme standard. If omitted, the standard must be diluted immediately prior to use and discarded after use.

## STOPPING REAGENT:

2% (w/v) Tris buffer (pH 10)

Dissolve 20 g of Tris buffer salt (**B-TRIS500**) in 900 mL of distilled water. Adjust the pH to 10 with 1 M NaOH and the volume to 1 L. Stable for > 2 years at room temperature.

## EQUIPMENT (RECOMMENDED):

1. Disposable plastic micro-cuvettes (1 cm light path, 3.0 or 1.5 mL), e.g. Plastibrand<sup>®</sup>, semi-micro, PMMA; Brand cat. no. 759115 ([www.brand.de](http://www.brand.de)).
2. Micro-pipettors, e.g. Gilson Pipetman<sup>®</sup> (50  $\mu$ L and 100  $\mu$ L).
3. Positive displacement pipettor, e.g. Eppendorf Multipette<sup>®</sup>
  - with 5.0 mL Combitip<sup>®</sup> (to dispense 0.1 mL substrate solutions).
  - with 25 mL Combitip<sup>®</sup> (to dispense extraction buffer and 3.0 mL of Stopping Reagent).
4. Analytical balance.
5. Spectrophotometer set at 400 nm.
6. Vortex mixer (e.g. IKA<sup>®</sup> Yellowline Test Tube Shaker TTS2).
7. Stop clock.
8. Bench centrifuge or Whatman GF/A glass fibre filter paper circles.

## CONTROLS AND PRECAUTIONS:

1. For each set of assays, a reagent blank value should be determined. To obtain this value, add 3.00 mL of Stopping Reagent to 0.10 mL of pre-equilibrated CellG5 reagent solution and then add 0.10 mL of diluted enzyme preparation. A single reagent blank determination is sufficient for each batch of assays.
2. If reagent blank absorbance value exceeds 0.2, then the CellG5 substrate should be discarded.
3. If reaction values exceed 1.2, then the enzyme preparation should be diluted in the appropriate buffer and re-assayed. Appropriate corrections to the calculations should then be made.
4. CellG5 is not hydrolysed by enzymes other than *endo*-cellulase (and *endo*-xylanase enzymes which have an *endo*-cellulase secondary activity), so it can be used to specifically assay for this enzyme in fermentation broths and industrial enzyme preparations. Although cellulases from all four organisms tested exhibit very similar standard curves in terms of their relative abilities to hydrolyse CellG5 versus CM-cellulose, it

is recommended that the user generate their own standard curve for their particular cellulase to ensure the most accurate determination of enzyme activity.

### USEFUL HINTS:

1. The substrate solution (Bottle 1) should be stored below  $-10^{\circ}\text{C}$  upon arrival and each of the four vials thawed prior to use as required to maximise the long term stability of the reagent.
2. The total number of assays which can be performed can be doubled by halving the volumes of all the reagents used and by employing 1.5 mL semi-micro spectrophotometer cuvettes. This increases the capacity of K-CellG5-2V from 60 to 120 assays and K-CellG5-4V from 120 to 240 assays. Do not alter the concentration of substrate in the final reaction mixture.

## A. MANUAL ASSAY PROCEDURE:

### Enzyme Extraction and Dilution:

1. Add 1.0 mL of liquid enzyme preparation to 49 mL of Extraction/Dilution buffer (100 mM, pH 4.5 or 6.0) using a positive displacement dispenser (these solutions can be very viscous) and mix thoroughly. This is termed the **Original Extract**.
2. If further dilution is required, add 1.0 mL of the **Original Extract** to 9.0 mL of Extraction/Dilution buffer (100 mM, pH 4.5 or 6.0) (10-fold dilution) and mix thoroughly. This process of dilution is repeated until a suitable concentration of cellulase for assay is achieved.
3. Alternatively, add 1.0 g of powder enzyme sample to 50 mL of Extraction/Dilution buffer (100 mM, pH 4.5 or 6.0) and gently stir the slurry over a period of approx. 15 min or until the sample is completely dispersed or dissolved. Clarify this solution by centrifugation (1,000 g, 10 min) or by filtration through Whatman No. 1 (9 cm) filter circles. This is termed the **Original Extract**.
4. If further dilution is required, add 1.0 mL of the **Original Extract** to 9.0 mL of Extraction/Dilution buffer (10-fold dilution) (100 mM, pH 4.5 or 6.0) and mix thoroughly. This process of dilution is repeated until a suitable concentration of cellulase for assay is achieved.

### Assay of Cellulase:

1. Dispense 0.10 mL aliquots of CellG5 substrate solution directly to the bottom of 13 mL glass tubes and pre-incubate the tubes at  $40^{\circ}\text{C}$  for approx. 3 min.

2. Pre-incubate diluted cellulase solution at 40°C for 3 min.
3. To each tube containing CellG5 solution, add 0.1 mL of cellulase solution to the bottom of the tube, stir on a vortex mixer and incubate tubes at 40°C for exactly 10 min (from time of addition).
4. At the end of the 10 min incubation period, add 3.0 mL of Stopping Reagent and stir the tube contents.
5. Read the absorbance of the reaction solutions and the reagent blank at 400 nm against distilled water.

## CALCULATION OF ACTIVITY:

Units of cellulase per mL or g of enzyme solution being assayed:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5 in one minute under the defined assay conditions, and is termed a **CellG5 Unit**.

CellG5 Units/mL or g of original enzyme preparation:

$$= \frac{\Delta A_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\epsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Vol.}} \times \text{Dilution}$$

where:

$\Delta A_{400}$  = Absorbance (reaction) - Absorbance (blank)

Incubation Time = 10 min

Total volume in cell = 3.2 mL (or 1.6 mL)

Aliquot assayed = 0.1 mL (or 0.05 mL)

$\epsilon_{mM}$  (4-nitrophenol) = 18.1 (at 400 nm) in 2% Tris buffer (pH 10)

Extraction volume = 50 mL per 1.0 mL or 1.0 g of original enzyme preparation

Dilution = Dilution of the original extract (if required)

Thus:

CellG5 Units/mL or g of original enzyme preparation:

$$= \frac{\Delta A_{400}}{10} \times \frac{3.2}{0.1} \times \frac{1}{18.1} \times \frac{50}{1} \times \text{Dilution}$$

$$= \Delta A_{400} \times 8.84 \times \text{Dilution}$$

## **B. AUTOMATED ASSAY PROCEDURE:**

### **EQUIPMENT (RECOMMENDED):**

1. ChemWell<sup>®</sup>-T auto-analyser fitted with a 405 nm filter.
2. Polypropylene tubes (13 mL capacity).
3. Pipettors, 1 mL (e.g. Gilson Pipetman<sup>®</sup>) to dispense enzyme extract.
4. Adjustable-volume dispenser: 0-10 mL (for Extraction Buffer).
5. Top-pan balance.
6. Vortex mixer (optional).
7. Stop Clock.
8. Microfuge or Whatman GF/A glass fibre filter paper circles (9 cm diameter).

### **PREPARATION OF REAGENTS:**

All reagents are prepared as described above for the manual assay procedure with the exception of the stopping reagent, which is described below.

### **PREPARATION OF STOPPING REAGENT:**

#### **500 mM Sodium Carbonate, pH 11.0.**

Dissolve 53 g of sodium carbonate (anhydrous) in 1 L of distilled water and adjust the pH to approx. 11.0. Store in a sealed bottle. Stable for ~ 3 months at room temperature.

### **ENZYME EXTRACTION AND ASSAY:**

1. The Original Extract is prepared as described above for the manual assay procedure.
2. Perform the assay using the **K-CellG5** ChemWell<sup>®</sup>-T assay file.

### **Automated Assay Parameters:**

<b>Assay volumes:</b>	CellG5 Reagent:	0.025 mL
	Sample (extract):	0.025 mL
	Stopping Reagent:	0.300 mL
<b>Reaction time:</b>	10 min at 37°C	
<b>Wavelength:</b>	405 nm	
<b>Assay type:</b>	Stopped reaction	
<b>Reaction direction:</b>	Increase	



## CALCULATION OF ACTIVITY (Automated Assay Procedure):

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5 in one minute under the defined assay conditions, and is termed a **CellG5 Unit**.

CellG5 Units/mL or g of original enzyme preparation:

$$= \frac{\Delta A_{405}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\epsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Weight}} \times \text{Dilution}$$

### where:

$\Delta A_{405}$  = Absorbance (reaction) - Absorbance (blank)

Incubation Time = 10 min

Total Volume in Cell = 0.350 mL

Aliquot Assayed = 0.025 mL

Apparent  $\epsilon_{mM}$  of *p*-nitrophenol (at 405 nm) in 500 mM sodium carbonate, pH 11 = 12.456

Extraction volume = 50 mL per 1 mL or 1 g (cellulase)

Dilution = Dilution of the extract including the original extraction (e.g. 50 mL per 1 mL or 1 g of cellulase)

### Thus:

CellG5 Units/mL or g of original enzyme preparation =

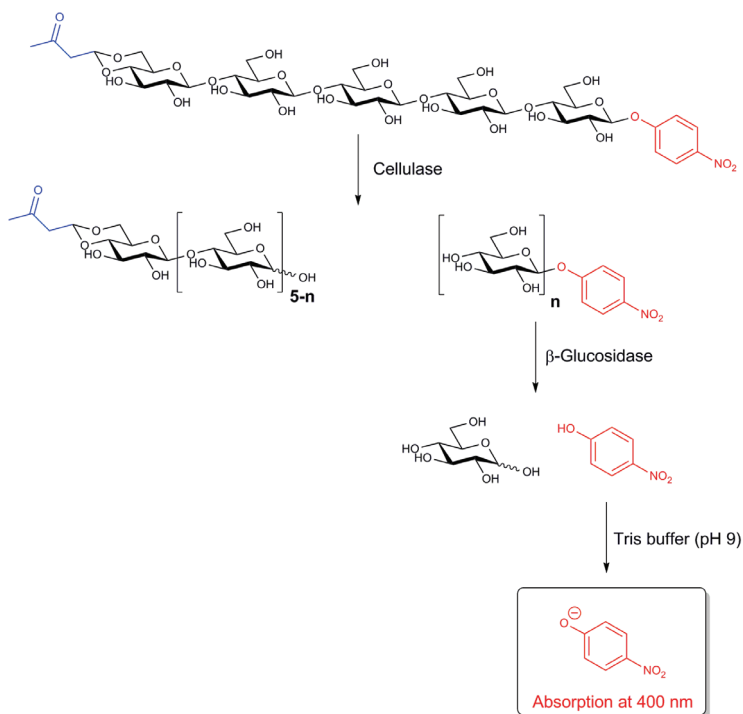
$$= \frac{\Delta E_{405}}{10} \times \frac{0.350}{0.025} \times \frac{1}{12.456} \times \text{Dilution}$$
$$= \Delta E_{405} \times 0.1124 \times \text{Dilution}$$

### NOTE:

The absorption coefficient ( $\epsilon_{mM}$ ) of 12.456 was determined experimentally from the absorbance obtained using a 50  $\mu$ M solution of 4-nitrophenol in 500 mM sodium carbonate in a ChemWell<sup>®</sup>-T Auto-Analyser system.

## APPENDIX:

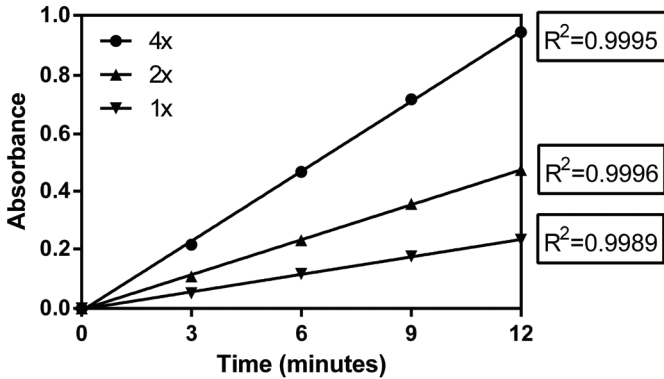
### A. Principle of the assay



**Scheme 1.** Theoretical basis of the CellG5 cellulase assay procedure.

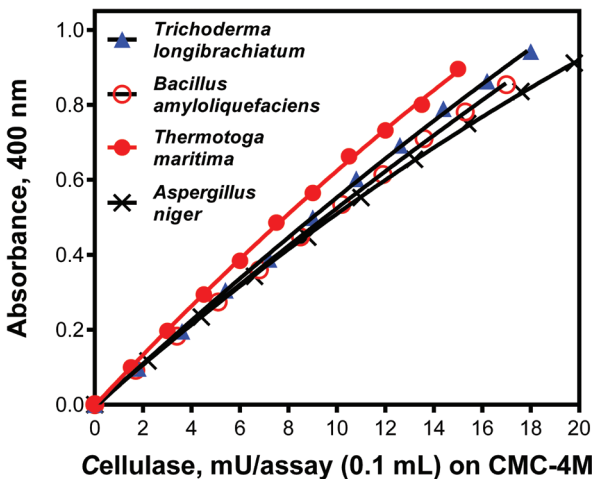
The CellG5 reagent contains two components; 1) 4,6-O-(3-Ketobutyridene)-4-nitrophenyl- $\beta$ -D-cellopentaoside (BPNPG5) and 2) thermostable  $\beta$ -glucosidase. The ketone blocking group prevents any hydrolytic action by the  $\beta$ -glucosidase on BPNPG5. Incubation with an *endo*-cellulase generates a non-blocked colourimetric oligosaccharide that is rapidly hydrolysed by the ancillary  $\beta$ -glucosidase. The rate of formation of 4-nitrophenol is therefore directly related to the hydrolysis of BPNPG5 by the *endo*-cellulase. The reaction is terminated and the phenolate colour is developed on addition of Tris buffer solution (pH 10).

## B. Linearity



**Figure 1.** Linearity of CellG5 assay with *Trichoderma longibrachiatum* cellulase (**E-CELTR**) in sodium acetate buffer (pH 4.5). Reaction was terminated at 3 min intervals by adding 2% Tris buffer solution (3.0 mL, pH 10).

## C. Relationship between cellulase hydrolysis of CellG5 and CMC-4M



**Figure 2.** Standard curve relating the activity of *Trichoderma longibrachiatum* (**E-CELTR**), *Aspergillus niger* (**E-CELAN**), *Bacillus amyloliquefaciens* (**E-CELBA**) and *Thermotoga maritima* (**E-CELTM**) cellulase on CM-cellulose 4M (Nelson-Somogyi) to absorbance increase at 400 nm on hydrolysis of CellG5.

Equations relating the action of several pure cellulases on CellG5 substrate and on CM-cellulose 4M (Nelson-Somogyi method) are shown below:

***Trichoderma longibrachiatum*** (assays run at 40°C)

Units on CMC-4M = 1.05 x CellG5 Units

***Bacillus amyloliquefaciens*** (assays run at 40°C)

Units on CMC-4M = 1.10 x CellG5 Units

***Thermotoga maritima*** (assays run at 80°C)

Units on CMC-4M = 0.93 x CellG5 Units

***Aspergillus niger*** (assays run at 40°C)

Note that the relationship between hydrolytic action on CellG5 and CMC-4M is appreciably non-linear for *A. niger* cellulase. The non-linear relationship can be accurately expressed as:

Units/mL or g on CMC-4M

$$= 0.0504 \times ((\Delta A_{400})^2 + 0.1709) \times (\Delta A_{400}) \times \frac{\text{Extraction Vol.}}{\text{Sample Vol.}} \times \text{Dilution}$$

**where:**

$\Delta A_{400}$  = Absorbance (reaction) - Absorbance (blank)

Extraction volume = 50 mL per 1.0 mL or 1.0 g of original enzyme preparation

Dilution = Dilution of the original extract (if required)

#### D. Repeatability and Reproducibility

The repeatability and reproducibility of the assay was determined by having two analysts perform a series of assays on three different samples ranging in activity from 55-164 mU/mL over two consecutive days. The results are outlined in Table 1.

**Table 1.**

<i>Trichoderma longibrachiatum</i> Cellulase	55 mU/mL $\Delta$ Abs (400 nm)	82 mU/mL $\Delta$ Abs (400 nm)	164 mU/mL $\Delta$ Abs (400 nm)
Day 1 <sup>A</sup>	0.320	0.471	0.925
Day 2 <sup>A</sup>	0.324	0.477	0.925
Day 1 <sup>B</sup>	0.305	0.464	0.910
Day 2 <sup>B</sup>	0.308	0.458	0.883
Standard Dev. ( $\sigma$ )	0.009	0.008	0.020
%CV	2.96	1.75	2.17

Note: A = Analyst 1, B = Analyst 2

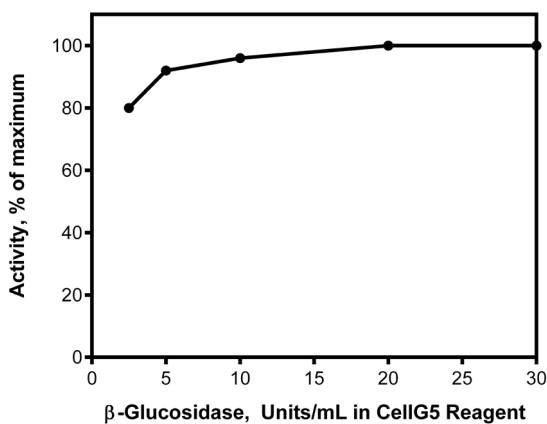
Note that the Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as  $3 \times \sigma$  of the blank sample solution absorbance and  $10 \times \sigma$  of the blank sample solution absorbance, respectively, using absorbance values from 10 replicates.

LOD =  $1.2 \times 10^{-3}$  U/mL (corresponding to an absorbance of 0.007)

LOQ =  $4.1 \times 10^{-3}$  U/mL (corresponding to an absorbance of 0.023)

### E. Effect of the concentration of thermostable $\beta$ -glucosidase in the reagent solution on determined endo-cellulase values

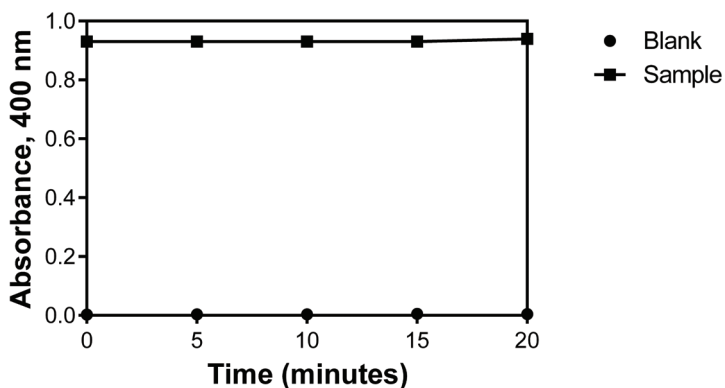
From the results shown in Figure 3, it is evident that the concentration of  $\beta$ -glucosidase required to saturate the reaction is 20 U/mL of CellG5 reagent solution.



**Figure 3.** The effect of the concentration of  $\beta$ -glucosidase in the substrate reagent solution on the determined absorbance values.

### F. Thermal stability of CellG5 reagent

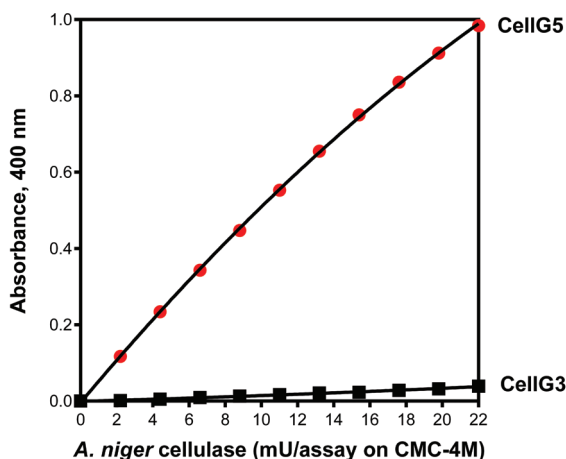
The stability of the CellG5 reagent solution was determined by incubating aliquots of this solution at  $60^\circ\text{C}$  for 0-20 min. These solutions were then used to assay the activity of the CellG5 standard solution (at  $40^\circ\text{C}$ ). From the data shown in Figure 4, it is evident that the reagent is very stable at  $60^\circ\text{C}$ . Over a 20 min incubation period, no difference could be detected in either the blank or sample absorbance values for the pre-incubated versus non-incubated CellG5 reagent solutions.



**Figure 4.** Investigation into the thermal stability of CellG5 reagent.

### G. Comparison of CellG5 reagent with CellG3

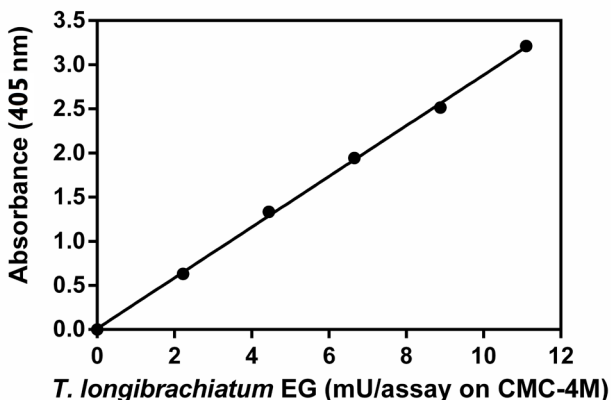
CellG5 is a superior reagent to CellG3, displaying higher sensitivity for all *endo*-cellulases investigated to date. The difference in sensitivity is most noticeable for *Aspergillus niger* cellulase as shown in Figure 5.



**Figure 5.** Comparison between the absorbance obtained upon incubation of *A. niger* cellulase with CellG5 and CellG3 reagents.

## H. Standard curve for automated assay on ChemWell®-T auto-analyser.

All prior appendices apply to the manual assay format. The ChemWell®-T auto-analyser automatically converts the absorbance obtained in an assay into the cellulase activity measurement (in CellG5 units) in the sample solution.



**Figure 6.** Standard curve relating the absorbance obtained for the automated CellG5 assay on a ChemWell®-T system to *endo*-cellulase activity on CMC-4M.

### REFERENCES:

1. McCleary, B. V., McKie, V. & Draga, A. (2012). Measurement of *endo*-1,4- $\beta$ -glucanase. "Methods in Enzymology" (Gilbert, H. J., Ed.), **Vol. 510**, pp 1-17.
2. Somogyi, M. (1952). Note on sugar determination. *J. Biol. Chem.*, **195**, 19-23.
3. McCleary B. V., Mangan, D., Daly, R., Fort, S., Ivory R. & McCormack, N. Novel substrates for the measurement of *endo*-1,4- $\beta$ -glucanase (*endo*-cellulase). *J. Carbohydr. Res.*, **385**, 9-17.



**Bray Business Park, Bray,  
Co. Wicklow,  
A98 YV29,  
IRELAND.**

**Telephone: (353.1) 286 1220**

**Facsimile: (353.1) 286 1264**

**Internet: [www.megazyme.com](http://www.megazyme.com)**

**E-Mail: [info@megazyme.com](mailto:info@megazyme.com)**

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