Lichenase (K-MBG4)
Procedure for ChemWell® 2910 Auto-Analyser

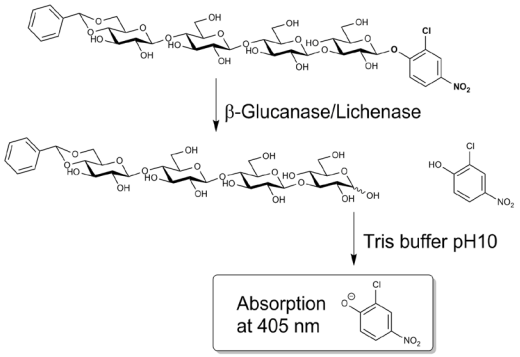
Requirements:

- Lichenase Assay Kit **(K-MBG4)** (provides ~ 330 assays).
- **K-MBG4 (LICHENASE)** ChemWell® 2910 assay file.
- Use in association with the Lichenase Assay Kit **(K-MBG4)** product data booklet.

Use:

A specific and sensitive colourimetric method for the determination of lichenase.

Assay Principle:



Procedure:

Prepare the assay reagents and use with the **K-MBG4 (LICHENASE)** ChemWell® 2910 assay file.

Lichenase Assay Kit Components:

Bottle 1: (x2) 4,6-*O*-benzylidene-2-chloro-4-nitrophenyl-β-(3[1]-β-D-cellotriosyl-glucose) (BCNPBG4) in 50% DMSO/H_2O (5 mL) plus sodium azide (0.02% w/v). Stable for > 4 years below -10ºC.

Bottle 2: Malt Flour Control 5 g (Malt β-glucanase activity ~ 0.13 MBG4 U/g; actual value stated on the vial label). Stable for > 4 years at room temperature.

Bottle 3: *Bacillus* sp. lichenase standard solution (5 mL; ~ 1.38 MBG4 U/mL; actual value stated on the vial label) in 50% aqueous glycerol plus sodium azide (0.02% w/v) and BSA (0.05% w/v). Stable for > 2 years below -10ºC.

Preparation of Kit Components:

1. The MBG4 reagent solution is used as provided. Store below -10ºC when not in use. Stable for > 2 days at room temperature.
2. Extraction of the β-glucanase present in the malt flour standard is performed as per the β-glucanase extraction procedure described on page 5. No further dilution is required.
3. With a positive displacement pipette, dispense 0.5 mL of the contents of bottle 3 to 9.5 mL of **Buffer D** and mix well. Once diluted, the standard is stable for at least 2 months below -10ºC.

Preparation of Reagents Not Supplied:

Stopping Reagent

(2% (w/v) Tris buffer, pH 10.0)

Dissolve 20 g of Tris buffer salt **(B-TRIS500)** in 900 mL of distilled water. Adjust the pH to 10.0 with 1 M NaOH and adjust the volume to 1 L. Stable for > 2 years at room temperature.

(C) Concentrated Phosphate Extraction/Dilution Buffer

(Sodium phosphate buffer, 0.5 M, pH 6.0)

Add 156 g of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) 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 4ºC.

(D) Phosphate Extraction/Dilution Buffer

(Sodium phosphate buffer, 100 mM, pH 6.5 containing 0.02% sodium azide)

Add 200 mL of concentrated phosphate buffer (C) to 750 mL of distilled water. Adjust the pH to 6.5 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 4ºC.

Enzyme Extraction and Dilution:

1. Add 1.0 mL of liquid enzyme preparation to 49 mL of **Buffer D** (100 mM sodium phosphate, pH 6.5) using a positive displacement dispenser (these solutions can be very viscous) and mix thoroughly. **This is termed the Original Extract.**

Alternatively:

Add 1.0 g of **powder enzyme** sample to 50 mL of **Buffer D** (100 mM sodium phosphate, pH 6.5) 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 x *g*, 10 min) or by filtration through Whatman No. 1 (9 cm) filter circles. **This is termed the Original Extract**.

2. Add 1.0 mL of the **Original Extract** to 9.0 mL of **Buffer D** (100 mM sodium phosphate, pH 6.5) (10-fold dilution) and mix thoroughly. This process of dilution should be repeated until a suitable concentration of lichenase for assay is achieved.
3. Perform the assay using the **K-MBG4 (LICHENASE)** ChemWell® 2910 assay file.

Assay Parameters:

Assay volumes:	MBG4 Reagent:	0.03 mL
	Sample:	0.06 mL
	Stopping Reagent:	0.210 mL
Reaction time:	10 min at 37ºC	
Wavelength:	405 nm	
Assay type:	Stopped reaction	
Reaction direction:	Increase	

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Calculation of Activity:

One Unit of activity is defined as the amount of enzyme required to release one micromole of 2-chloro-4-nitrophenol (CNP) from MBG4 in one minute under the defined assay conditions, and is termed an **MBG4 Unit.**

MBG4 Units/mL or g of original enzyme preparation:

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

where:

ΔE_{405}	= Absorbance (reaction) - Absorbance (blank)
Incubation Time	= 10 min
Total Volume in Cell	= 0.3 mL
Aliquot Assayed	= 0.06 mL
ε_{mM} of *p*-nitrophenol (at 405 nm) in 2% Tris buffer, pH 10	= 13.273
Extraction Volume	= 8 mL per 0.5 g of malt flour
D	= Dilution of the original extract

Thus:

$$= \frac{\Delta E_{405}}{10} \times \frac{0.3}{0.06} \times \frac{1}{13.273} \times \frac{50}{1} \times D$$

$$= \Delta E_{405} \times 1.884 \times D$$

MBG4 Units/mL or g:

NOTE:
The absorption coefficient (ε_{mM}) of 13.273 was experimentally determined under the conditions of the automated MBG4 Lichenase assay using a ChemWell® 2910 auto-analyser.

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