MALT β-GLUCANASE (K-MBG4)
Procedure for ChemWell® 2910 Auto-Analyser

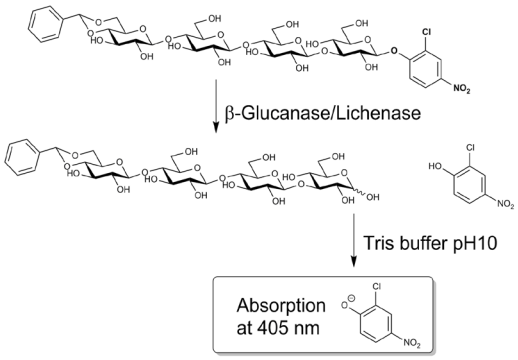
Requirements:

- Malt β-Glucanase Assay Kit **(K-MBG4)** (provides ~ 400 assays).
- **K-MBG4 (BLANK)** and **K-MBG4 (SAMPLE)** ChemWell® 2910 assay files and the **K-MBG4 (CALC)** ChemWell® 2910 indices file.
- Use in association with the Malt β-Glucanase Assay Kit **(K-MBG4)** product data booklet.

Use:

A specific and sensitive colourimetric method for the determination of malt β-glucanase in malt flour.

Assay Principle:



Procedure:

Prepare the assay reagents and use with the **K-MBG4 (BLANK)** and **K-MBG4 (SAMPLE)** ChemWell® 2910 assay files and the **K-MBG4 (CALC)** ChemWell® 2910 indices file.

Malt β-Glucanase Assay Kit Components:

Bottle 1: (x2) 4,6-*O*-benzylidene-2-chloro-4-nitrophenyl-β-(3¹-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.

(A) Concentrated Acetate Buffer

(Sodium acetate buffer, 1 M, pH 4.5)

Add 60 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 0.02% w/v 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 0.2 g of sodium azide and dissolve. Stable for > 1 year at 4ºC.

Enzyme Extraction and Dilution:

1. Mill malt (approx. 20 g sample) to pass a 0.5 mm screen using a Tecator Cyclotec® mill or equivalent.
2. Accurately weigh 0.5 g samples of malt flour into glass centrifuge tubes (14 x120 mm; 17 mL capacity).
3. Add 8.0 mL of **Extraction Buffer B** (100 mM sodium acetate, pH 4.5) to each tube and stir the contents thoroughly on a vortex mixer.
4. Allow the enzyme to extract over a 15 min period at room temperature (less than 30ºC), with occasional mixing.
5. a) Filter the turbid suspension through glass fiber filter paper to obtain the malt β-glucanase extract solution **or**

   b) centrifuge the tubes and contents at 3,000 x *g* for 5 min and carefully pipette off the malt β-glucanase extract solution.
6. Perform the assay using the **K-MBG4 (SAMPLE), K-MBG4 (BLANK)** ChemWell® 2910 assay files and the **K-MBG4 (CALC)** ChemWell® 2910 indices file.

Assay Parameters:

Assay volumes:	MBG4 Reagent:	0.02 mL
	Sample:	0.08 mL
	Stopping Reagent:	0.200 mL
Reaction time:	10 min at 37ºC	
Wavelength:	405 nm	
Assay type:	Stopped reaction	
Reaction direction:	Increase	

Megazyme, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland
t + 353 1 286 1220 e info@megazyme.com w www.megazyme.com

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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 = 0.39 mL

Aliquot Assayed = 0.06 mL

ε_{mM} of *p*-nitrophenol (at 405 nm) in 2% Tris buffer, pH 10 = 14.552

Extraction Volume = 8 mL per 0.5 g of malt flour

D = Dilution of the original extract

Thus:

$$= \frac{\Delta E_{405}}{10} \times \frac{0.3}{0.08} \times \frac{1}{14.552} \times \frac{8}{0.5} \times D$$

$$= \Delta E_{405} \times 0.412 \times D$$

MBG4 Units/mL or g:

NOTE:
The absorption coefficient (ε_{mM}) of 14.552 was experimentally determined under the conditions of the automated MBG4 Malt β-Glucanase assay using a ChemWell® 2910 auto-analyser.

Megazyme, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland
t + 353 1 286 1220 e info@megazyme.com w www.megazyme.com