

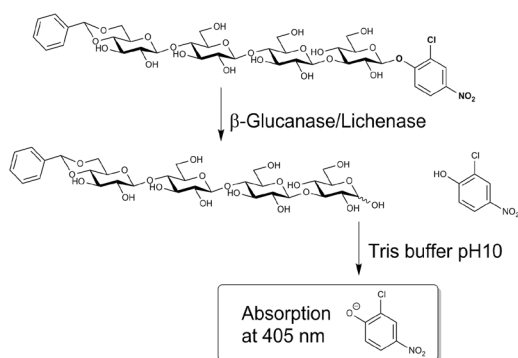
### Requirements:

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

### Use:

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

### Assay Principle:



### Procedure:

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

### Malt $\beta$ -Glucanase Assay Kit Components:

**Bottle 1: (x2)** 4,6-O-benzylidene-2-chloro-4-nitrophenyl- $\beta$ -(3<sup>1</sup>- $\beta$ -D-cellobiosyl-glucose) (BCNPBG4) in 50% DMSO/H<sub>2</sub>O (5 mL) plus sodium azide (0.02% w/v). Stable for > 4 years at -20°C.

**Bottle 2:** Malt Flour Control 5 g (Malt  $\beta$ -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 at -20°C.

### Preparation of Kit Components:

1. The MBG4 reagent solution is used as provided. Store at -20°C when not in use. Stable for > 2 days at room temperature.
2. Extraction of the  $\beta$ -glucanase present in the malt flour standard is performed as per the  $\beta$ -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 at -20°C.

### 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 4°C.

### 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 30°C), with occasional mixing.
5. a) Filter the turbid suspension through glass fiber filter paper to obtain the malt  $\beta$ -glucanase extract solution **or**  
b) centrifuge the tubes and contents at 3,000 x g for 5 min and carefully pipette off the malt  $\beta$ -glucanase extract solution.
6. Perform the assay using the **K-MBG4 (SAMPLE)**, **K-MBG4 (BLANK)** ChemWell®-T assay files and the **K-MBG4 (CALC)** ChemWell®-T indices file.

### Assay Parameters:

**Assay volumes:** MBG4 Reagent: 0.025 mL  
Sample: 0.125 mL  
Stopping Reagent: 0.250 mL

**Reaction time:** 10 min at 37°C  
**Wavelength:** 405 nm  
**Assay type:** Stopped reaction  
**Reaction direction:** Increase





# MALT $\beta$ -GLUCANASE (K-MBG4) Procedure for ChemWell®-T Auto-Analyser

## 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:

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

## where:

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

Incubation Time = 10 min

Total Volume in Cell = 0.4 mL

Aliquot Assayed = 0.125 mL

$\epsilon_{\text{mM}}$  of *p*-nitrophenol (at 405 nm) in 2% Tris buffer, pH 10  
= 12.456

Extraction Volume = 8 mL per 0.5 g of malt flour

D = Dilution of the original extract

## Thus:

$$= \frac{\Delta E_{405}}{10} \times \frac{0.4}{0.125} \times \frac{1}{12.456} \times \frac{8}{0.5} \times D$$
$$= \Delta E_{405} \times 0.411 \times D$$

MBG4 Units/mL or g:

## NOTE:

The absorption coefficient ( $\epsilon_{\text{mM}}$ ) of 12.456 was experimentally determined under the conditions of the automated MBG4 Malt  $\beta$ -Glucanase assay using a ChemWell®-T auto-analyser.

