**β-Amylase (Betamyl® Method; K-BETA3)**

**Procedure for ChemWell®-T Auto-Analyser**

### Requirements:
- β-Amylase Assay Kit (K-BETA3) (provides ~ 500 assays).
- Use in association with the β-Amylase Assay Kit (K-BETA3) product data booklet.

### Use:
A specific colourimetric method for the measurement of β-amylase, especially in cereals such as malt or barley.

### Assay Principle:

#### Procedure:

1. Prepare the assay reagents and use with the K-BETA3 ChemWell®-T assay file.

### β-Amylase Assay Kit Components:

**Bottle 1:** (x2) Each vial contains p-nitrophenyl-β-D-maltotrioside (PNPβ-G3) plus β-glucosidase (50 U) and stabilisers. Stable for > 4 years at -20°C.

**Bottle 2:** Tris/HCl buffer (25 mL, 1 M, pH 8.0) plus disodium EDTA (20 mM) and sodium azide (0.02% w/v). Stable for approx. 4 years at 4°C.

**Bottle 3:** MES buffer (48 mL, 1 M, pH 6.2) plus disodium EDTA (20 mM), BSA 10 mg/mL and sodium azide (0.10% w/v). Stable for approx. 4 years at 4°C.

**Bottle 4:** Cysteine hydrochloride (16 g). Stable for > 2 years at room temperature.

**Bottle 5:** Malt flour of standardised β-amylase activity (as specified on bottle label). Stable for > 4 years at room temperature.

### Preparation of Kit Components:

1. Dissolve the contents of bottle 1 in 10 mL of boiled and cooled distilled water. This is Betamyl-3® Substrate Solution. Divide into appropriately sized aliquots and store in polypropylene tubes at -20°C between use. Store unused reagent on ice awaiting use. Do not dissolve the contents of the second bottle until required. Stable for > 2 years at -20°C.

2. Dilute 2.5 mL of the contents of bottle 2 to 50 mL with distilled water. Before use, add 0.88 g of cysteine HCl (bottle 4; Megazyme cat. no. G-LCYST200) (final concentration of cysteine HCl approx. 100 mM) and adjust the pH to 8.0 with 4 M NaOH. Stable for 8 h at 4°C.

3. Dilute the entire contents of bottle 3 to 500 mL with distilled water. Stable for ~ 1 year at 4°C.

4. Use as supplied. Stable for > 4 years at room temperature.

5. Use the contents of bottle 5 as supplied. Stable for > 4 years at room temperature.

### Preparation of Stopping Reagent (Not supplied):

1% (w/v) Tris buffer, pH ~ 8.5.

Dissolve 10 g of Tris buffer salts (Megazyme cat. no. B-TRIS500) in 900 mL of distilled water. Adjust the pH to 8.5 (if necessary) and the volume to 1 litre. Stable for ~ 1 year at room temperature.

### Extraction and Assay of Milled Grain Samples:

1. Mill malt or barley to pass a 0.5 mm screen with a suitable laboratory mill (e.g. Buhler Miag disc mill DLFU; Setting fine, or Frisch Pulverisette 14® with 0.5 mm screen).

2. To exactly 0.5 g of flour in a 13 mL polypropylene tube (Sarstedt cat. no. 60.541.685 PP; www.sarstedt.com), add 5.0 mL of Extraction Buffer (Bottle 2).

3. Allow the enzyme to extract over a 1 hour period at room temperature, with frequent vigorous stirring on a vortex mixer (approx. 5 times over the 1 hour period). Alternatively, place the tube into a Stuart Blood Tube Rotator which allows end-over-end mixing and run the machine for 1 hour.

4. Filter an aliquot of the enzyme preparation through Whatman GF/A glass fibre filter paper, or centrifuge in a bench or micro-centrifuge at a minimum of 2,000 g for 10 min.

5. Add 0.2 mL of filtrate to 4.0 mL of Dilution/Assay Buffer B (Bottle 3), mix, and use this for the assay of β-amylase activity.

β-Amylase (Betamyl® Method; K-BETA3) Procedure for ChemWell®-T Auto-Analyser

Assay Parameters:

Assay volumes:
- Betamyl-3® Reagent: 0.020 mL
- Sample: 0.020 mL
- Stopping Reagent: 0.300 mL

Reaction time: 10 min at 37°C
Wavelength: 405 nm
Assay type: stopped reaction
Reaction direction: increase

Calculation of Activity:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable β-glucosidase, required to release one micromole of \( p \)-nitrophenol from PNPβ-G3 in one minute under the defined assay conditions, and is termed a Betamyl-3® Unit.

Betamyl-3® Units/g flour:

\[
\text{Betamyl-3® Units/g flour} = \Delta E_{400} \times \frac{1}{E_{\text{mm}}^\text{M}} \times \frac{1}{\text{Sample Weight}} \times \frac{5}{\text{Extraction Vol.}} \times \frac{21}{D}
\]

where:
- \( \Delta E_{400} \): Absorbance (reaction) - Absorbance (blank)
- Incubation Time: 10 min
- Total Volume in Cell: 0.34 mL
- Aliquot Assayed: 0.02 mL
- \( E_{\text{mm}}^\text{M} \): \( E_{\text{mm}} \) of \( p \)-nitrophenol (at 405 nm) in Tris buffer, pH 8.5 = 12.8
- Extraction Volume: 5 mL per 0.5 gram (milled grain sample)
- \( D \): 0.2 to final volume of 4.2 mL (i.e. 21 fold).

Thus:

Betamyl-3® Units/g milled grain:

\[
\Delta E_{400} \times \frac{0.34}{10} \times \frac{1}{0.02} \times \frac{12.8}{12.8} \times \frac{5}{0.5} \times 21 = \Delta E_{400} \times 27.9
\]

NOTE:
The absorption coefficient \( E_{\text{mm}} \) of 12.8 was experimentally determined under the conditions of the automated β-amylase assay using a ChemWell®-T auto-analyser.