Preparation of Kit Components:

1. Dissolve the entire contents of one of Bottle 1 in 8.0 mL of freshly boiled and cooled distilled water. Divide into 2-3 mL aliquots and store frozen between use. At 0-5°C the dissolved substrate is stable for 7 days; in the frozen state it is stable for at least 12 months.

2. Dilute the entire contents of Bottle 2 (50 mL) (plus a crystalline precipitate which may be present) to 1 L with distilled water before use. The pH should be 5.4; adjust if necessary. Stable at 0-5°C for 12 months.

3. Milled wheat of standardised α-amylase activity (as specified on the vial label). Use as supplied in the extraction and assay procedure. It is recommended that the user standardises at least one batch of their own wheat flour to be employed as a secondary reference flour.

Preparation of Stopping Reagent (Not supplied):

500 mM Sodium Carbonate, pH 11.0.
Dissolve 53 g of sodium carbonate (anhydrous) in 1 litre of distilled water and adjust the pH to approx. 11.0. Store in a sealed bottle to prevent the formation of carbonate. Stable at room temperature for at least 3 months.

Extraction and Assay of Milled Grain Samples:

1. Mill wheat, barley or other grain (approx. 10-50 g sample) to pass a 0.5 mm screen (e.g. with a Fritsch centrifugal mill).

2. Accurately weigh 0.5 g of milled grain into a polypropylene tube of 13 mL capacity.

3. To each tube add 4.0 mL of Extraction Buffer solution (pH 5.4) and stir the flask contents vigorously for approx. 20 sec.

4. Immediately centrifuge an aliquot of the extract (e.g. 2 x 1 mL) at 11,000 g for 3 min in a microfuge.

5. Use the clear supernatant in the automated Amylase SD assay procedure using a ChemWell® 2910 auto-analyser. Assay the enzyme activity within 2 h.


Assay Parameters:

- Assay volumes:
  - Amylase SD Reagent: 0.025 mL
  - Sample: 0.075 mL
  - Stopping Reagent 2: 0.100 mL

- Reaction time: 5 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 α-glucosidase, required to release one micromole of p-nitrophenol from EtPNPG7 in one minute under the defined assay conditions, and is termed an Amylase SD Unit.

Amylase SD Units/g milled grain:

where:

\[
\text{Amylase SD Units/g milled grain} = \frac{\Delta E_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{E_{\text{mM}}} \times \frac{\text{Extraction Vol.}}{\text{Sample Weight}} \times D
\]

\[
\Delta E_{400} = \text{Absorbance (reaction) - Absorbance (blank)}
\]

Incubation Time = 5 min
Total Volume in Cell = 0.2 mL
Aliquot Assayed = 0.075 mL

\[
E_{\text{mM}} \text{ of } p\text{-nitrophenol (at 405 nm) in 500 mM sodium carbonate, pH 11} = 10.508
\]

Extraction Volume = 4 mL per 0.5 gram (milled grain sample)
D = Dilution of the original extract (if required)

Thus:

Amylase SD Units/g milled grain:

\[
\text{Amylase SD Units/g milled grain} = \frac{\Delta E_{400}}{5} \times \frac{0.2}{0.075} \times \frac{1}{10.508} \times \frac{4}{0.5}
\]

\[
= \Delta E_{400} \times 0.406
\]

NOTE:
The absorption coefficient \(E_{\text{mM}}\) of 10.508 was experimentally determined under the conditions of the automated Amylase SD assay using a ChemWell® 2910 auto-analyser.

Reference: