Amylase SD (K-AMYLSD)
Procedure for ChemWell®-T Auto-Analyser

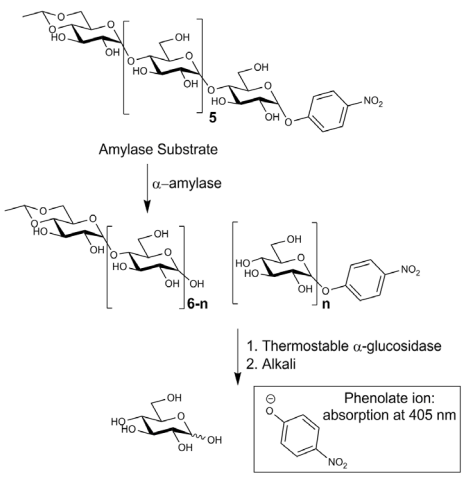
Requirements:

- Amylase SD Assay Kit **(K-AMYLSD)** (provides ~ 640 assays).
- **K-AMYLSD (BLANK)** and **K-AMYLSD (SAMPLE)** ChemWell®-T assay files and the **K-AMYLSD (CALC)** ChemWell®-T indices file.
- Use in association with the Amylase SD Assay Kit **(K-AMYLSD)** product data booklet.

Use:

A highly sensitive colourimetric method for the determination of α-amylase in sprout damaged wheat grain (also known as pre-harvest sprouting or weather damaged wheat grain) and "late maturity α-amylase" wheat grain.

Assay Principle:



Procedure:

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

Amylase SD Assay Kit Components:

Bottle 1: (x2) Amylase SD Reagent. Lyophilised powder. Stable for > 5 years below -10°C.

Bottle 2: Concentrated Extraction Buffer (50 mL). Stable for > 4 years at room temperature.

Bottle 3: Milled Wheat Control. Amylase SD activity as shown on the vial label. Stable for > 4 years at room temperature.

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 **8.0 mL of Extraction Buffer solution** (pH 5.4) and allow the enzyme to extract over 10 min at 40°C with occasional mixing.
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®-T auto-analyser. **Assay the enzyme activity within 2 h.**
6. Perform the assay using the **K-AMYLSD (SAMPLE), K-AMYLSD (BLANK)** ChemWell®-T assay files and the **K-AMYLSD (CALC)** ChemWell®-T indices file.

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Assay Parameters:

Assay volumes:

Amylase SD Reagent:	0.025 mL
Sample:	0.075 mL
Stopping Reagent 2:	0.100 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 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:

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

where:

ΔE_{405}	= Absorbance (reaction) - Absorbance (blank)
Incubation Time	= 10 min
Total Volume in Cell	= 0.2 mL
Aliquot Assayed	= 0.075 mL
E_{mM} of *p*-nitrophenol (at 405 nm) in 500 mM sodium carbonate, pH 11	= 12.345
Extraction Volume	= 8 mL per 0.5 gram (milled grain sample)
D	= Dilution of the original extract (if required)

Thus:

Amylase SD Units/g milled grain:

$$= \frac{\Delta E_{405}}{10} \times \frac{0.2}{0.075} \times \frac{1}{12.345} \times \frac{8}{0.5}$$

$$= \Delta E_{405} \times 0.346$$

NOTE: The absorption coefficient (E_{mM}) of 12.345 was experimentally determined under the conditions of the automated Amylase SD assay using a ChemWell®-T auto-analyser.

Reference:

McKie, V. A. & McCleary, B. V. (2015). A rapid, automated method for measuring α-amylase in pre-harvest sprouted (sprout damaged) wheat. *Journal of Cereal Science,* 64, 70-75.

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