Limit-Dextrinase (K-PullG6) Procedure for ChemWell®-T Auto-Analyser

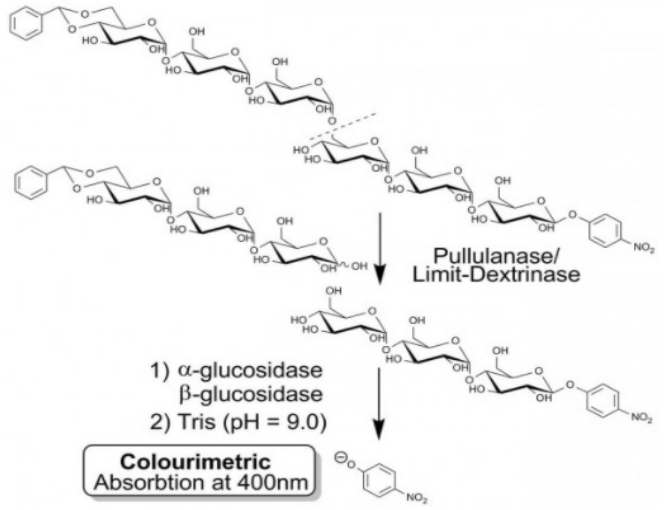
Requirements:

- Limit-Dextrinase Assay Kit **(K-PullG6)** (provides ~ 330 assays).
- **K-PullG6 (Limit-Dextrinase)** ChemWell®-T assay file.
- Use in association with the Limit-Dextrinase Assay Kit **(K-PullG6)** product data booklet.

Use:

A specific and sensitive colourimetric method for the determination of Limit-dextrinase.

Assay Principle:



Procedure:

Prepare the assay reagents and use with the **K-PullG6 (Limit-Dextrinase)** ChemWell®-T assay file.

Limit-Dextrinase Assay Kit Components:

Bottle 1: (x2) 4,6-*O*-benzylidene-4-nitrophenyl-6^3-α-D-maltotriosyl-maltotriose (BPNPG3G3) plus thermostable α and β-glucosidase. Stable for > 5 years at -20ºC.

Bottle 2: Not required for Limit-Dextrinase assay.

Bottle 3: Not required for Limit-Dextrinase assay.

Bottle 4: Control malt flour of standardised limit-dextrinase activity, ~ 0.28 U/g on PullG6 substrate (exact activity stated on vial label). Stable for > 4 years; store sealed below -10ºC.

Preparation of Kit Components:

1. Dissolve the contents of one bottle 1, in 5 mL of distilled water. This is the **PullG6 Reagent Solution**. Divide into aliquots and store in polypropylene tubes below -10ºC between use and on ice during use. Do not dissolve the contents of the other bottle until required. Once dissolved, the reagent is stable for > 2 years below -10ºC

3. Dilute the contents of bottle 3 to 500 mL with distilled water. This is **Buffer B**. Store at 4ºC. Stable for > 2 years at 4ºC.

4. The limit-dextrinase extraction/activation procedure for the premilled standard is exactly the same as that described for barley samples on page 7 of the data booklet.

Preparation of Additional Reagents & Stopping Reagent

Stopping Reagent

(2% (w/v) Tris buffer, pH 9.0)

Dissolve 20 g of Tris buffer salt **(B-TRIS500)** in 900 mL of distilled water. Adjust the pH to 9.0 with 1 M NaOH and adjust the volume to 1 L. Stable for > 2 years at room temperature.

(C) Dilution Buffer

(Sodium maleate (100 mM, pH 5.5) plus sodium azide, 0.02% w/v)

Dissolve maleic acid (5.8 g, Sigma cat. no. M0375) in 400 mL of distilled water and adjust the pH to 5.5 with sodium hydroxide solution (2 M) (requires about 35 mL). Add sodium azide (0.1 g) and readjust the pH to 5.5. Adjust the volume to 500 mL. Store at room temperature. Stable for > 2 years at room temperature.

(D) Extraction/Activation Buffer

(Sodium maleate (100 mM, pH 5.5) plus sodium azide, 0.02% w/v plus 25 mM dithiothreitol)

Prepare immediately before use, add dithiothreitol (0.1 g) to 25 mL of Buffer C.

Enzyme Extraction and Dilution:

1. Mill barley (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 flour into a 13 mL polypropylene tube.

3. To each tube add 8.0 mL of **Buffer D**, mix the contents well and close the screw-cap tightly.

4. Allow the enzyme to extract over 5 h at 40°C, vortexing for ~ 10 sec once per h.

5. Filter an aliquot of the solution through a Whatman GF/A glass fibre filter paper, or centrifuge an aliquot at 1,000 g for 10 min.
   Assay enzyme activity within 2 h.

Procedure:

Perform the assay using the **K-PullG6 (Limit-Dextrinase** ChemWell®-T assay file.

Assay Parameters:

Assay volumes:	PullG6 Reagent:	0.03 mL
	Sample:	0.03 mL
	Stopping Reagent:	0.15 mL
Reaction time:	30 min at 37ºC	
Wavelength:	405 nm	
Assay type:	Stopped reaction	
Reaction direction:	Increase	

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Pullulanase (K-PullG6)
Procedure for ChemWell®-T Auto-Analyser

Calculation of Activity:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase and β-glucosidase, required to release one micromole of 4-nitrophenol from BPNPG3G3 in one minute under the defined assay conditions, and is termed a PullG6 Unit (PU)

where:

ΔE_{405}	= Absorbance (reaction) - Absorbance (blank)
Incubation Time	= 30 min
Total Volume in Cell	= 0.21 mL
Aliquot Assayed	= 0.03 mL
ε_{mM} of *p*-nitrophenol (at 405 nm) in 2% Tris buffer, pH 9	= 12.456
Extraction Volume	= 8 mL per 0.5 g
D	= Dilution of the original extract

Thus:

Limit-Dextrinase Units/g of milled malt:

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

$$= \frac{\Delta E_{405}}{30} \times \frac{0.21}{0.03} \times \frac{1}{12.456} \times \frac{8}{0.5} \times D$$

$$= \Delta E_{405} \times 0.300 \times D$$

NOTE:
The absorption coefficient (ε_{mM}) of 12.456 was experimentally determined under the conditions of the automated PullG6 Limit Dextrinase assay using a ChemWell®-T auto-analyser.

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