α-Amylase (Ceralpha® Method; K-CERA) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- α-Amylase Assay Kit **(K-CERA)** (provides ~ 1000 assays).
- **K-CERA WHEAT** or **K-CERA MALT** or **K-CERA MICRO** ChemWell®-T assay file.
- Use in association with the α-Amylase Assay Kit **(K-CERA)** product data booklet.

Use:

The Ceralpha® Method employing the Amylase HR Reagent mixture is a specific colourimetric method used to measure cereal, fungal and bacterial α-amylase activity.

Procedure:

Prepare the assay reagents and use with the **K-CERA WHEAT** (for wheat or barley samples) or **K-CERA MALT** (for malt samples) or **K-CERA MICRO** (for microbial samples) ChemWell®-T assay file.

α-Amylase Assay Kit Components:

Bottle 1: Concentrated Extraction Buffer A. Store at room temperature.

Bottle 2: Concentrated Stopping Reagent. Store at room temperature.

Bottle 3: Malt flour of standardised α-amylase activity (as specified on bottle label). Store at room temperature.

Bottle 4: (x2) Amylase HR Reagent mixture. Freeze dried BPNPG7 plus thermostable α-glucosidase. Stable for > 4 years at -20°C.

Preparation of Kit Components:

1. Dilute the entire contents (50 mL) (plus a crystalline precipitate which may be present) to 1000 mL with distilled water before use. Stable at 0-5°C for 12 months. The pH should be 5.4; adjust if necessary.
2. Dilute the entire contents (25 mL) to 500 mL with distilled water. Stable at room temperature for 3 months.
3. Use the Malt flour control, as supplied, in procedure A.
4. Dissolve the entire contents of one vial in 10 mL of 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.

Preparation of Extraction Buffer B (for bacterial α-amylase) (Not supplied):

Maleic acid (Sigma M0375; 0.1 M)	23.2 grams/2 litres
Sodium chloride	11.6 grams/2 litres
Calcium chloride dihydrate (2 mM)	0.6 grams/2 litres
Sodium azide (Sigma S2002; 0.01% w/v)	0.2 grams/2 litres

Add the maleic acid and sodium chloride to 1600 mL of distilled water and adjust the pH to 6.5 with 4 M sodium hydroxide. Add the calcium chloride and sodium azide and adjust the volume to 2 L. Store at room temperature between use.
Use this buffer directly without further dilution.

Some bacterial α-amylases are unstable on dilution. This problem is usually resolved by inclusion of BSA (0.5 mg/mL) in the buffer.

Extraction and Assay:

A. Wheat and Barley Flours:

1. Mill wheat, barley or other grain (approximately 10-50 g sample) to pass a 0.5 mm screen (e.g. with a Fritsch centrifugal mill).
2. Accurately weigh 3.0 g of flour into a flask of 50 mL capacity.
3. To each flask add 20.0 mL of Extraction Buffer solution (pH 5.4) and stir the flask contents vigorously.
4. Allow the enzyme to extract over 15-20 min at 40°C with occasional mixing.
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.
6. Perform the assay using the **K-CERA WHEAT** ChemWell®-T assay file.

B. Malt Flours:

1. Mill malt (20 g sample) to pass a 0.5 mm screen.
2. Accurately weigh 0.5 g malt flour into a 100 mL volumetric flask.
3. To the volumetric flask add a solution of 1% sodium chloride plus 0.02% calcium chloride plus 0.02% sodium azide; adjust to volume.
4. Allow the enzyme to extract for 15-20 min at room temperature, with occasional stirring.
5. Filter an aliquot of the solution through a Whatman GF/A glass fibre filter paper, or centrifuge at 1,000 *g* for 10 min.
6. Dilute 0.5 mL of the filtrate with 9.5 mL of Extraction Buffer Solution. **Assay enzyme activity** within 2 h.
7. Perform the assay using the **K-CERA MALT** ChemWell®-T assay file.

C. Microbial Preparations:

i. Liquid preparations:

1. Add 1 mL of liquid enzyme preparation (using a positive displacement dispenser) to **Buffer A or B** (49 mL, pH 5.4 or 6.5) and mix thoroughly. This is termed the **Original Extract.**
2. Dilute 1.0 mL of **Original Extract** 10-fold by addition to 9.0 mL of appropriate **Buffer (A or B)** and mix thoroughly. Repeat this step until a dilution suitable for assay is obtained. For example, for the industrial enzyme preparation, Bacterial Alpha-Amylase (from Kerry Ingredients, Ireland) a dilution of the **Original Extract** of approximately 4,000-fold is required.

   NOTE: The dilution factor used here must be applied to the result that is generated by the assay.
3. Perform the assay using the **K-CERA MICRO** ChemWell®-T assay file.

ii. Powder preparations:

1. Add 1 g of enzyme powder preparation to 50 mL of **Buffer A or**

Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland
t + 353 1 286 1220 e info@megazyme.com w www.megazyme.com

α-Amylase (Ceralpha® Method; K-CERA) Procedure for ChemWell®-T Auto-Analyser

B (pH 5.4 or 6.5) and gently stir the slurry over a period of about 15 min or until the sample is completely dispersed or dissolved.

2. Clarify this solution (the **Original Extract**) by centrifugation (1,000 *g*, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles.
3. Dilute 1.0 mL of this solution 10-fold by addition to 9.0 mL of appropriate **extraction/dilution buffer** and mix thoroughly. Repeat this step until a dilution suitable for assay is obtained.

   NOTE: The dilution factor used here must be applied to the result that is generated by the assay.
4. Perform the assay using the **K-CERA MICRO** ChemWell®-T assay file.

Assay Parameters:

Assay volumes:

Ceralpha® Reagent:	0.020 mL
Sample:	0.020 mL
Stopping Reagent:	0.300 mL

Reaction time: 20 min **(K-CERA WHEAT)**
10 min **(K-CERA MALT & K-CERA MICRO)**
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 BPNPG7 in one minute under the defined assay conditions, and is termed a **Ceralpha® Unit**.

Ceralpha® Units/g flour:

$$= \frac{\Delta E_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Weight}} \times D$$

where:

ΔE_{400}	= Absorbance (reaction) - Absorbance (blank)
Incubation Time	= 10 min (malt and microbial extracts) = 20 min (wheat and barley extracts)
Total Volume in Cell	= 0.34 mL
Aliquot Assayed	= 0.02 mL
ε_{mM} of *p*-nitrophenol (at 405 nm) in 1% tri-sodium phosphate, pH 11	= 12.8
Extraction Volume	= 20 mL per 3 gram (wheat and barley) = 100 mL per 0.5 gram (malt) = 50 mL per 1 g or 1 mL (microbial)
D	= Dilution of the original extract = 20-fold (malt extracts)

NOTE:
The absorption coefficient (ε_{mM}) of 12.8 was experimentally determined under the conditions of the automated α-amylase assay using a ChemWell®-T auto-analyser.

Thus:

A. For Wheat and Barley:

Ceralpha® Units/g flour:

$$= \frac{\Delta E_{400}}{20} \times \frac{0.34}{0.02} \times \frac{1}{12.8} \times \frac{20}{3.0}$$

$$= \Delta E_{400} \times 0.443$$

B. For Malt:

Ceralpha® Units/g milled malt:

$$= \frac{\Delta E_{400}}{10} \times \frac{0.34}{0.02} \times \frac{1}{12.8} \times \frac{100}{0.5} \times 20$$

$$= \Delta E_{400} \times 531.25$$

C. For Microbial preparations:

Ceralpha® Units/mL or g of original preparation:

$$= \frac{\Delta E_{400}}{10} \times \frac{0.34}{0.02} \times \frac{1}{12.8} \times \frac{50}{1} \times D$$

$$= \Delta E_{400} \times 6.641 \times D$$

Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland
t + 353 1 286 1220 e info@megazyme.com w www.megazyme.com