

Micro Assay for Flour Alpha Amylase Activity

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Adapted by Mary Guttieri for the Soft Wheat Quality Laboratory

The new method adapts the AACC Method 22-02 using the Ceralpha K-CERA (Megazyme) alpha-amylase assay procedure for higher throughput to determine flour alpha-amylase activity in a microwell plate. All reagents, controls and precautions are as described in the Megazyme manual.

Required Materials

- Ceralpha Alpha-Amylase Kit (AACC Method 22-02)
- 50 mL conical centrifuge tubes
- Centrifuge with rotor to spin 50 mL conical tubes at 1000 xg
- Analytical balance
- Microplate reader and plate (510 nm)
- Vortex mixer
- Water bath at 40°C
- Multichannel repeating pipette

Ceralpha Substrate and Stopping Reagent

Ceralpha substrate is prepared as described and stored frozen (-20°C) in 1 mL aliquots in microcentrifuge tubes.

Additional Stopping Reagent is prepared using 1% w/v sodium phosphate tribasic dodecahydrate in distilled water adjusted to pH 11.

Enzyme Extraction

1. Accurately weigh 3.0 g of ground grain or flour into a 50 mL conical centrifuge tube.
2. Add 20.0 mL of 1X Extraction Buffer solution (pH 5.4) to each tube and mix vigorously.
3. Allow enzyme to extract over 20 minutes in a 40°C water bath, with occasional mixing.
4. Centrifuge 1,000 x g for ten minutes.
5. Assay enzyme activity within two hours.

Reaction Blank

A single set of triplicate Reaction Blanks (non-enzymatic control) is prepared as follows for each batch of samples being analyzed.

1. 0.3 mL of stopping reagent
2. 20 µL of substrate solution at the start of the reaction time
3. 20 µL of any enzyme preparation in the sample set

The mean absorbance of the non-enzymatic control is subtracted from all assays conducted during that

day to establish the background or blank absorbance.

Assay Procedure

1. Dispense 20 μL aliquots of Ceralpha Reagent Solution into a microtiter plate and pre-incubate the tubes and contents at 40°C for 5 min. Dispense 3 aliquots for each enzyme extract (assay each extract in triplicate).
2. To each well containing Ceralpha Reagent solution (20 μL), add 20 μL of wheat α -amylase extract directly to the bottom of the well at 30 second intervals.
3. Incubate at 40°C for exactly twenty min from time of addition.
4. Following the 20 min incubation period, add exactly 0.3 mL of Stopping Reagent.
5. Read the absorbance of the solutions and the reaction blank at 400 nm against 340 μL distilled water.