

Protein Extraction From Cereal Flour

Procedure

- 1) Weigh 50 mg of flour into a 1.5 ml microfuge tube.
- 2) Add 1 ml of Borate extraction buffer and vortex to suspend the flour.
- 3) Incubate with shaking at 37°C for at least 2 h (up to overnight)
- 4) Centrifuge at room temperature 15 min.
- 5) Remove supernatant to a new tube. This solution can be used directly as a total protein extract for SDS-PAGE, blotting etc. 10 µl is a good amount to use and is ~25-30 µg of protein for maize endosperm extracts.
- 6) To fractionate the zein proteins (prolamins) from other proteins, precipitate the other proteins with 70% ethanol. Add 300 µl of total protein extract to 700 µl of ethanol, mix well and incubate ~ 2 h.
- 7) Centrifuge 15 min at room temperature. Remove supernatant to a new tube and dry in a SpeedVac, this pellet contains the extracted zein proteins (prolamins). The zein pellet can be resuspended in 200 µl of ddH₂O.
- 8) Wash the 70% ethanol pellet with 70% ethanol 2 times and partially air-dry the pellet until the edges become transparent. Resuspend this pellet in 200 µl of Laemmli sample buffer, 8 M urea or IPG rehydration buffer. (NEVER heat samples resuspended in urea or rehydration buffer!!! Can you say carbamylation?)
- 9) To check the samples by 1-D SDS-PAGE 5 µl is a good amount to load. For 2-D SDS-PAGE 10 µl is a good amount, or measure protein concentrations to load by protein mass.

Solutions

Borate Extraction Buffer	IPG Rehydration Buffer
12.5 mM Sodium Borate	8 M Urea
1% SDS	2% CHAPS
2% β-mercaptoethanol (add just before use)	20 mM DTT (Add just before use)
pH 10	

Adapted from Wallace, J.C., et al. (1990) Plant Physiology. 92(1): 191-196.