

## Proteins and peptides

- 5-10  $\mu\text{g}$  of pure protein/peptide is necessary for accurate composition and quantitative data analysis.
- Use reagents and solvents of the highest purity available.
- Buffers, detergents, glycerol, trace metals and salts can interfere with analysis. These reagents should either be removed from samples prior to analysis or reduced to minimal.
- Avoid presence of amino-containing substances (Tris) since it will react with ninhydrin.
- Put the protein/peptide into polyethylene or polypropylene micro centrifuge tubes. The sample volume should be  $< 200 \mu\text{l}$ .
- Avoid lyophilizing the sample. This can lead to substantial loss of sample for some proteins. It is more practical to concentrate the sample in a speed-vacuum.
- Always wear gloves (powder free) and work in a clean dust-free area. Dust and unprotected hands are the major sources of amino acid contamination.

## Sample extraction protocols for analysis of plant tissues

*Important: samples must be extracted by the client*

### **A) Free amino acids determination (methanol:chloroform:water extraction protocol)** (Hacham et al., *Plant Physiology* 128: 454-462, 2002)

- Freeze  $\sim 150$  mg of tissue in liquid nitrogen.
- Grind to a fine powder with a precooled mortar and pestle in the presence of  $600 \mu\text{l}$  of water:chloroform:methanol (3:5:12 v/v). Do not let the tissue thaw.
- Transfer to 1.5 ml tube and centrifuge at full speed for 2 minutes.
- Collect the supernatant and place in 2 ml tube.
- Re-extract the residue with another  $600 \mu\text{l}$  of water:chloroform:methanol extraction buffer.
- Centrifuge at full speed for 2 minutes.
- Collect the supernatant and combine with the previous one in the 2 ml tube.
- Add  $300 \mu\text{l}$  of chloroform and  $450 \mu\text{l}$  of water to the combined supernatants.
- Centrifuge at full speed for 2 minutes.
- Collect the upper water:methanol phase and transfer to a fresh tube.

- Speed vac to dry (about 3 hours).
- Keep at -20 until you submit to Facility.

**B) Total protein determination (TCA/acetone extraction)**

- 1- Freeze ~150 mg of tissue in liquid nitrogen.
- 2- Grind to a fine powder with a precooled mortar and pestle. Do not let the tissue thaw.
- 3- Suspend the powder in 10% TCA/acetone (w/v) (5-15 ml solution per gram tissue). Add  $\beta$ -mercaptoethanol to a final concentration of 2% (vol/vol) immediately before use.
- 4- Store at  $-20^{\circ}\text{C}$  for at least 1 h. Proteins should precipitate as white flakes.
- 5- Centrifuge at 5.000 g for 30 min at  $4^{\circ}\text{C}$ . Carefully pipette out the supernatant and discard.
- 6- Add 10 ml ice-cold acetone to the pellet.
- 7- Store at  $-20^{\circ}\text{C}$  for at least 1 h
- 8- Centrifuge for 10 min at 5.000 g at  $4^{\circ}\text{C}$ . Carefully remove the supernatant with a pipette and discard.
- 9- Repeat steps 6-8 two more times.
- 10- Dry the pellet at room temperature to eliminate the acetone fully. The pellet should ideally be white or very light colored.
- 11- Keep at -20 until you submit to Facility.