**Proteins and peptides**

- 5-10 µg 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 µl.
- 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)

1- Freeze ~150 mg of tissue in liquid nitrogen.

2- Grind to a fine powder with a precooled mortar and pestle in the presence of 600 µl of water:chloroform:methanol (3:5:12 v/v). Do not let the tissue thaw.

3- Transfer to 1.5 ml tube and centrifuge at full speed for 2 minutes.

4- Collect the supernatant and place in 2 ml tube.

5- Re-extract the residue with another 600 µl of water:chloroform:methanol extraction buffer.

6- Centrifuge at full speed for 2 minutes.

7- Collect the supernatant and combine with the previous one in the 2 ml tube.

8- Add 300 µl of chloroform and 450 µl of water to the combined supernatants.

9- Centrifuge at full speed for 2 minutes.

10- Collect the upper water:methanol phase and transfer to a fresh tube.

11- Speed vac to dry (about 3 hours).

12- 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 β-mercaptoethanol to a final concentration of 2% (vol/vol) immediately before use.

4- Store at −20 °C for at least 1 h. Proteins should precipitate as white flakes.

5- Centrifuge at 5.000 g for 30 min at 4 °C. Carefully pipette out the supernatant and discard.

6- Add 10 ml ice-cold acetone to the pellet.

7- Store at −20 °C for at least 1 h

8- Centrifuge for 10 min at 5.000 g at 4 °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.