

2-D – Gel Electrophoresis

Guidelines for Sample Submission and Preparation

Sample Submission

Samples for 2-D-gel electrophoresis may be submitted:

- In Solution (acceptable solvents are listed below, maximum submission volume is 100 microliter)
- Lyophilized

Submitted samples will be dissolved in/diluted with lysis solution (see below).

Precipitated samples will be solubilized in lysis solution and clarified by centrifugation if necessary. The solution or supernatant will be analyzed. Incompatible components (see list below) should be removed from the sample prior to submission. A summary on removal techniques is available at the facility.

Samples containing incompatible components will be subjected to protein precipitation (\$15 per sample).

This will result in a delay in sample processing.

Radioactively labeled samples cannot be processed.

Protein Amounts and Volume: *must* be specified on the request form

Minimum: 200 microgram of total protein. If in solution, submit in a maximum of 100 microliter volume.

Maximum: 1 mg total protein. If in solution submit in a maximum of 100 microliter volume.

The sample should NOT contain:

Salts

Buffers other than Tris

Other small ionic molecules

Ionic detergents (e.g. SDS)

Nucleic acids

Polysaccharides

Lipids

Phenolic compounds

Insoluble material

The following components are ACCEPTABLE:

Water

Urea

Thiourea

Non-ionic or zwitterionic detergents (e.g. CHAPS, NP-40, Triton X-100)

Tris base

Reducing agents (e.g. DTT, TBP)

Lysis Solution:

8-9 M urea or 7M urea/2M thiourea

CHAPS 2-4% (w/v) or NP-40 or Triton X-100

40mM Tris

20-100mM DTT or 2mM TBP (tributylphosphine)

Protease inhibitor if necessary, e.g. complete mini (Roche) 1 tablet/10mL buffer

General Hints for Sample Preparation

- Add protease inhibitor immediately after or during cell lysis.
- In case protein extraction requires components not compatible with 2D electrophoresis, remove these components prior to submission e.g. by precipitating and re-dissolving in lysis solution (see summary on removal techniques).
- Samples containing SDS must be diluted to a final concentration of 0.25% SDS or less. Adjust the protein concentration accordingly.

Questions? Contact the Protein Structure Facility at:

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