



BIOTECHNOLOGY CENTER

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Guidelines for Sample Submission and Preparation for 2-D Electrophoresis

Sample Submission

Samples for 2D-gel electrophoresis may be submitted:

- ◆ In Solution : maximum sample volumes: 50 μ l (7 cm IPG strip; mini gel)
100 μ l (13 cm IPG strip; large gel)

(acceptable solvents are listed below)
- ◆ 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.

Contaminants (see list below) should be removed from the sample prior to
submission. A summary on removal techniques is available at the facility.

Protein Amounts: must be specified; maximum of 1 mg protein per sample.

Radioactively labeled samples cannot be processed.

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
Lipids



Protein Sciences Facility

315 Noyes Laboratory • 505 S. Mathews Ave. • Urbana, IL 61801

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<http://www.life.uiuc.edu/biotech>



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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/10 ml 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).
- ◆ SDS containing samples must be diluted to a final concentration of 0.25% SDS or less. Adjust the protein concentration accordingly.



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