



2 Dimensional Electrophoresis Sample Preparation Guidelines

Sample preparation is a critical element in achieving optimal 2D electrophoresis separation results. Hence, discussions with an APAF staff member concerning the nature of samples to be sent for analysis is highly recommended.

Sample Preparation

Where possible it is advisable that samples are sent to APAF in their original form. For example as frozen tissue or dried down pellets. Where this is not possible samples should be supplied free of the following components which interfere with the first dimension isoelectric focusing step:

- salt
- nucleic acids
- polysaccharides
- lipids

Salt is a particular problem which leads to burning of IPG strips and we recommend the following desalting methods:

- Methanol precipitation
- TCA/acetone precipitation
- Spin columns e.g. Centricon
- Dialysis

Note: If additional work needs to be done at APAF to remove contaminating components from samples then additional costs will be incurred.

Amount of Material

The following table provides a guideline to the amounts of protein required for various gel types:

Analytical load (fluorescent stain) mini gel	10-50 µg total protein
Analytical load (fluorescent stain) large gel	100-200 µg total protein
Preparative (coomassie) load mini gel	100-500 µg total protein
Preparative (coomassie) load	1-2 mg total protein

large gel	
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If you are unsure of protein concentrations and need to know how much sample to send, the following is an **approximate** guide:

- Bacteria are approximately half their dry-weight in protein
- Plasma/Serum is approximately 60mg/mL protein
- 1×10^7 Mammalian cells is approximately equal to 1mg protein
- Human tears contain 1 μg of protein in volumes between 0.2 μL -2.0 μL
- Mammalian tissue (eg rat liver) requires a minimum of 10mg freeze dried material
- Human Urine anywhere between 0.5mL to 10mL needed for a large analytical gel ("normal" control people will require 10mL).

Permits

If you are sending any biological samples to APAF from countries outside of Australia go to the [Quarantine Guidelines](#) or [contact us](#) in regards to permits needed for Australian quarantine regulations and the recommended couriers to use.

Pathogenicity

Biological samples coming into APAF should be accompanied by documentation of potential pathogenicity or pathogen free status otherwise APAF will presume all samples from human and animal origin are potential pathogens and will be treated accordingly.

How do I send samples from Overseas to APAF?

For guidelines and documentation required visit our [Quarantine guidelines](#).

Sample Request Forms

Once you have followed all the guidelines you can [download](#) the 2D Protein Electrophoresis Request Form.