

Sample clean-up: Nucleic acid

Sample type: Cell, Tissue, Blood

Method: Nuclease treat & Centrifugation

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1. The presence of nucleic acid makes the sample viscous and can cause streaking in the acidic region.
2. High molecular weight nucleic acid can be removed by centrifugation.
3. Nucleases are often employed during sample preparation, particularly with microbial samples with high nucleic acid to protein ratios.
4. Things to bear in mind when using nucleases:
 - DNases require Mg^{2+} .
 - Most nucleases are inactive in high urea.
 - Nucleases can show up in the gel image as extra spots.

Methods to remove nucleic acids from sample:

- Enzymatic digestion: simplest method for removal of DNA; sample preparation can be achieved in a single step, by the addition of the enzyme prior to loading the first-dimension IPG. Adding endonuclease to the sample after solubilization at high pH (40 mM Tris) allows efficient digestion of nucleic acids while minimizing the action of contaminating proteases.
- Ultra-centrifugation: large nucleic acids will sediment and polycations such as polyethyleneimine are added to disrupt protein-nucleic acid interactions; protein loss is high.
- Mechanical disruption: ultrasonic probes and bead mills are effective tools for shearing nucleic acids into fragments that are too small to interfere with IEF.