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2D Electrophoresis - Sample Cleanup

Prior to IEF, remove contaminating salts, buffers, and other chemicals from samples by dialysis, precipitation, or buffer exchange. A protocol for buffer exchange using Bio-Rad's Micro Bio-Spin™ P-6 columns is provided here. Another alternative is the use of the ReadyPrep 2-D cleanup kit to effectively precipitate sample protein and remove contaminants. It has the additional benefit of concentrating the sample to a desired volume.

Buffer Exchange (Desalting)

Bio-Rad's Micro Bio-Spin columns are suitable for use with 1.5 or 2.0 ml microcentrifuge tubes and are completely autoclavable. They accommodate volumes of 20–75 µl; volumes less than 20 µl may affect recovery. The gel in the Micro Bio-Spin columns is suspended in either SSC buffer, pH 7.0, or Tris-HCl buffer, pH 7.4. For 2-D electrophoresis, it is best to exchange the sample into the 2-D sample solution (7 M urea, 2 M thiourea, 4% CHAPS) using the following protocol. DTT and ampholytes are added after the buffer exchange procedure.

1. Invert the column sharply several times to resuspend the settled gel and remove any bubbles. Snap off the tip and place the column in a 2.0 ml microcentrifuge tube (included). Remove the top cap. If the column does not begin to flow, push the cap back on the column and then remove it again to start the flow. Allow the excess packing buffer to drain by gravity to the top of the gel bed (about 2 min). Discard the drained buffer, then place the column back into the 2.0 ml tube.

2. Centrifuge for 2 min in a microcentrifuge at 1,000 × g to remove the remaining packing buffer. Discard the buffer.

3. Apply the new buffer in 500 µl aliquots. After each application, let the buffer drain out by gravity, then centrifuge the column at 1,000 × g for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat as required. Three washes result in >99% of the buffer exchanged. Four washes result in >99.9% of the buffer exchanged.

4. Place the column in a clean 1.5 or 2.0 ml microcentrifuge tube. Carefully apply the sample (20–75 µl) directly to the center of the column. Application of more or less than the recommended sample volume may decrease column performance.

5. Centrifuge the column for 2–4 min at 1,000 × g. Following centrifugation, the purified sample is in the new buffer. Molecules smaller than the column's exclusion limit are retained by the column.

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