ZOOM® IEF Fractionator

QUICK REFERENCE CARD

Catalog nos. ZF10001, ZF10002

25-0619 A 041003

Instructions are provided below for fractionating your sample using the ZOOM® IEF Fractionator. For detailed instructions, refer to the manual supplied with the product or download the manual from our Web site (www.invitrogen.com).

Preparing Samples

A brief procedure for preparing rat liver lysate is described below. For more details on sample preparation, see the manual.

1. To 50 mg (wet weight) of rat liver tissue, add the following:

   0.9 ml 1.1X IEF Denaturant (7.7 M urea, 2.2 M thiourea, 4.4% CHAPS, see manual for recipe).

   10 µl 100X protease inhibitor cocktail (containing 4-2-Aminoethyl-benzene sulfonyl-fluoride, EDTA, Bestatin, Pepstatin A, E-64, Leupeptin, see manual for details)

   10 µl 2 M DTT
2. Sonicate the sample on ice for 10 rounds of 20 seconds each, at ~50% power.
3. Add ~10 µl 1 M Tris base (the final pH should be 8.4-8.6).
4. Incubate the lysate for 30 minutes at room temperature.
5. Add 5.2 µl 99% N,N-Dimethylacrylamide (DMA) for alkylation.
6. Incubate the lysate for 30 minutes at room temperature.
7. Add 10 µl 2 M DTT to quench alkylation.
8. Incubate the lysate for 5 minutes at room temperature.
9. Centrifuge the lysate at 16,000 x g for 20 minutes at room temperature.
10. Proceed to **Diluting Samples for IEF** after determining the protein concentration of lysate or store the lysate at -80°C in small aliquots.

To determine the protein concentration of the lysate, use the Bradford protein assay method with BSA as the standard. The protein concentration of the lysate should be 8-9 mg/ml.

Diluting Samples for IEF

Dilute the reduced and alkylated sample prepared as described above to 0.6 mg/ml for IEF. You will need ~ 3.5 ml of the diluted sample for IEF using the ZOOM® IEF Fractionator.

1. To prepare 1 ml of diluted sample, mix the following:

   67-75 µl reduced and alkylated lysate (8-9 mg/ml)

   0.9 ml 1.1X IEF Denaturant

   10 µl ZOOM® Carrier Ampholytes, pH 3-10

   10 µl 2 M DTT

   Trace bromophenol blue dye

   Adjust to a final volume of 1.0 ml with deionized water.

   pH of the sample should be 7.2 (do not adjust the pH).
2. Use the diluted samples for IEF. Proceed to **Assembling ZOOM® IEF Fractionator**.

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Assembling ZOOM® IEF Fractionator

Instructions are provided below to assemble the ZOOM® IEF Fractionator using the complete set of 6 disks to obtain fractionation over a wide pH range of 3-10. If you are assembling the ZOOM® IEF Fractionator for the first time and for more details on the assembly, see the manual.

If you wish to assemble the ZOOM® IEF Fractionator with less than 6 disks to obtain fractionation over a narrow pH range, use Spacers instead of disks. See the manual for a detailed protocol.

1. Insert Sample Chamber Caps on top of the Sample Chambers through the port provided.

2. Place the Sample Chamber O-ring Seal (O-ring) on the groove of each Sample Chamber.

3. Holding the Chamber Assembly Tube in a vertical position, slide the Anode End Sealer into the Chamber Assembly Tube until the Anode End Sealer is flush with the top of the tube.
4. Place the assembled Sample Chamber (O-ring side down) from Step 2 on top of the Anode End Sealer. Gently push the Sample Chamber until the chamber is flush with the top of the tube.

5. Remove one ZOOM® Disk pH 3.0 from the package with forceps. Blot off excess packaging buffer from the disk by tapping the edge of the disk on filter paper. Place ZOOM® Disk pH 3.0 on the chamber using forceps.

6. Place the second assembled Sample Chamber from Step 2 (O-ring side down) on the disk. Slide the second Sample Chamber until it is flush with the top of the tube.
7. Remove one ZOOM® Disk pH 4.6 from the package with forceps. Blot off excess packaging buffer on filter paper. Place ZOOM® Disk pH 4.6 on the second chamber using forceps.

8. Repeat Steps 4-7 until all Sample Chambers are serially connected and are separated by 6 disks of respective pH placed in the following order:

   anode (+) end pH 3.0, pH 4.6, pH 5.4, pH 6.2, pH 7.0, pH 10.0 cathode (-) end

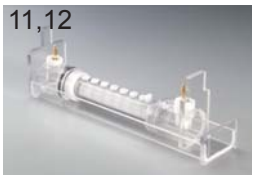
   Note: The first and last chambers are blank chambers (without disks).

Assembling ZOOM® IEF Fractionator, Continued

9. Place an O-ring on the groove of the Cathode End Sealer. Attach the Cathode End Sealer (O-ring on the groove facing down) to the last Sample Chamber.

10. Screw the Cathode End Screw Cap at the end of the Chamber Assembly Tube.

11. Slide the Chamber Assembly Tube into the Spill Trough and push until the tube fits into the Cathode Reservoir.
12. Place the assembled ZOOM® IEF Fractionator in a horizontal position on a benchtop. Proceed to **Loading Samples and Buffers**.



Loading Samples and Buffers

Load samples and buffers into the ZOOM® IEF Fractionator as described below. For more details, see the manual.

1. To prepare 20 ml of Anode Buffer, mix the following reagents:

ZOOM® Urea	8.4 g
ZOOM® Thiourea	3.0 g
Novex® IEF Anode Buffer (50X)	3.3 ml

   Adjust pH to 3.0 with Novex® IEF Anode Buffer (50X). Adjust the volume to 20 ml with deionized water.

2. To prepare 20 ml of Cathode Buffer, mix the following reagents:

ZOOM® Urea	8.4 g
ZOOM® Thiourea	3.0 g
Novex® IEF Cathode Buffer pH 3-10 (10X)	2.0 ml
Deionized Water	to 20 ml

   The pH should be 10.4. Do not adjust the pH.

3. Load 17.5 ml cathode buffer from Step 1 into the Cathode Reservoir of the Chamber Assembly Tube through the inlet provided (see figure A) using a sterile pipette.
4. Load 17.5 ml anode buffer from Step 2 into the Anode Reservoir through the inlet provided using a sterile pipette.

5. Remove the Sample Chamber Caps and add 670 µl of diluted protein sample (from Step 2, Diluting Samples for IEF) to each Sample Chamber through the inlet provided on top of the Sample Chamber (see figure B). **Do not add any sample to the blank Sample Chambers.**

Loading Samples and Buffers, Continued

6. Insert the Sample Chamber Caps on the Sample Chambers.
7. Place the lid on the assembled fractionator.
8. With the power supply turned off, connect electrode cords to the power supply, turn on the power, and proceed to **Performing IEF**.

Performing IEF

1. If the power supply has a current and power limiting capability, set a current limit at 2 mA and a power limit at 2 W.
2. Perform IEF using the following conditions:

   100 V for 20 minutes

   200 V for 80 minutes

   600 V for 80 minutes
3. While the run is in progress, label five sterile 1.5 ml microcentrifuge tubes with the appropriate pH range.
4. At the end of the run, turn off the power, disconnect cables from the power supply, and proceed to **Disassembling ZOOM® IEF Fractionator**.

Disassembling ZOOM® IEF Fractionator

Instructions are provided below to disassemble the ZOOM® IEF Fractionator. For more details on the disassembly, see the manual.

1. Remove lid from the ZOOM® IEF Fractionator.
2. Remove the Sample Chamber Caps from the Sample Chamber.
3. Using a 1.0 ml pipet tip on a pipettor, retrieve the fractionated sample from each Sample Chamber and transfer the sample to an appropriate 1.5 ml microcentrifuge tube from Step 3, above. Repeat to remove sample from all Sample Chambers.
4. To perform an **optional** washing step to retrieve any sample adhered to the Sample Chambers, wash the chambers as described in the manual.
5. Pull out the Chamber Assembly Tube from the Spill Trough. Using a pipette, discard anode and cathode buffers into a waste reservoir.
6. Unscrew the Cathode End Screw Cap at the end of the Chamber Assembly Tube.
7. Push the protrusion on the Anode End Sealer with your thumb to slide out Sample Chambers and Cathode End Sealer from the cathode end of the Chamber Assembly Tube.
8. Disassemble Sample Chambers and clean various parts of the fractionator. Discard the disks. Do not re-use ZOOM® Disks.
9. Store the fractionated samples at -80°C or proceed to the downstream application of choice.

If you are having problems using the ZOOM® IEF Fractionator, see the Troubleshooting section in the manual.

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This product is covered by Limited Use Label License No. 143. See the ZOOM® IEF Fractionator manual for detailed information.

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