SOP: SP008

Running of IEF strips by IPGphor

Materials and Reagents:

1. Protein sample, 100 to 400 μg
2. Water, Burdick & Jackson HPLC-grade
3. Dithiothreitol (DTT)
4. Rehydration buffer, 1 ml stock (note 1)
5. BCA protein assay reagents (note 2)
6. Amicon Ultra-15 5,000 MWCO unit
7. Benchtop centrifuge
8. Sterile bottle, 125 ml
9. Eppendorf tube, sterile, 1.7 ml
10. Pipetman, P-10
11. Pipet tips, 10 μl
12. Lyophilizer (note 3)
13. Pan sonicator
14. Pipetman, P-200
15. Pipet tips, 200 μl
16. Refrigerator, 4^{o}C
17. Ceramic strip holders, 7 cm or 13 cm
18. IPG Dry Strip, pH 4-7, 7 cm or 13 cm
19. Pipetman, P-1000
20. Pipet tips, 1000 μl
21. Dry Strip cover fluid
22. Kim wipes
23. IPGphor unit

Protocol:

1. _____ Transfer protein sample to a Amicon Ultra-15 5,000 MWCO filter unit.

2. _____ Centrifuge at 2,500 x g, 4^{o}C until volume is 1 ml.

3. _____ Decant eluent into a sterile bottle and add 15 ml of water to the filter unit.

4. _____ Repeat steps 2 to 3 three times (note 4).

5. _____ Transfer concentrate to a sterile 1.7 ml eppendorf centrifuge tube.

6. _____ Quantitate protein concentration using the BCA assay.

7. _____ Transfer required amount of protein to a sterile 1.7 ml eppendorf (note 5).

8. _____ Completely dry the protein using the lyophilizer.

9. _____ Remove from lyophilizer when sample is completely dry.

10. _____ Add 280 mg of DTT to a 1 ml stock of Rehydration buffer.

11. _____ Pan sonicate Rehydration buffer until DTT is completely in solution.

12. _____ Add Rehydration buffer to protein sample (note 6).

13. _____ Pan sonicate until protein is completely in solution.

14. _____ Place sample at 4^{o}C overnight (note 7).

15. _____ Transfer sample to ceramic strip holder using P200 Pipetman, keeping the solution between the two contact leads.

16. _____ Remove the plastic backing from a IPG Dry Strip (note 8).

17. _____ Place the strip in the ceramic strip holder, gel side down, taking care to avoid any air bubbles between the strip and the sample (note 9).

18. _____ Add 750 ml of Dry Strip Cover Fluid to the ceramic strip holder.

19. _____ Place plastic top on the ceramic strip holder.

20. _____ Carefully wipe outside of ceramic strip holder with a Kim wipe to remove any excess cover fluid.

21. _____ Turn on IPHphor unit and place ceramic strip holder on contact plates (note 10).

22. _____ Shut lid and select protocol number one.

23. _____ Edit parameters for either 7 cm strips (note 11) or 13 cm strips (note 12).

24. _____ Push the stop button when finished editing program parameters.

25. _____ Enter the number of strips being run.

26. _____ Start the program running (note 13).

27. _____ When the program is finished, open the lid, remove the ceramic strip holders, and begin preparing the strip for running second dimension gels (note 14).

28. _____ Wipe the contact plate free of excess cover fluid with a Kim wipe (note 15).

29. _____ Close lid and turn off IPGphor unit.

Notes:

1. Rehydration buffer is made as follows (for 25 ml):

	Final concentration	amount
De-ionized Urea	8 M	12 g (to deionize, add AG-501-X8 [BioRad] at 5 g/ 100 ml, and stir for 2hr at RT; filter)
CHAPS	2%	0.5 g
IPG buffer	0.5%	125 µl (stock solution)
Bromophenol blue	dark blue	a few grains
Water, B & J		to 25 ml

Make 1 ml aliquots in sterile 1.7 ml eppendorf centrifuge tubes and freeze at –20^{o}C until ready to use.

2. See BCA Protein Assay SOP SP003.
3. See Lyophilization SOP SP004.
4. It is essential to have the protein samples as clean and as free from ions as possible.
5. For a 7 cm silver stain gel (second dimension) 100 µg of protein is required; for a 7 cm Coomassie stain or a 13 cm silver stain 200 µg of protein; for a 13 cm Coomassie stain 400 µg of protein.
6. Use 128 µl of buffer for a sample that is being run on a 7 cm strip and 250 µl for a sample on a 13 cm strip.
7. Overnight works well for most samples, but for difficult samples the time may be increased to 48 hours.
8. IPG Dry Strips are stored at –20^{o}C prior to use. After removing the plastic backing take care not to touch the exposed gel strip to anything except the protein sample.

9. Take care to make sure the strip is correctly oriented, and the gel strip touches both contact leads. Any bubbles between the rehydrated protein and the gel strip will prevent transfer and separation of the proteins.
10. Make sure the anodes and cathodes of the strip holders and contact plate match up correctly. Use the guides on the contact plate for placement of 7 cm and 13 cm strip holders.
11. The program parameters for the 7 cm strips are:

a.	Rehydration	0:00	
b.	Running parameters	15°C	150 μA/strip
c.	S1 Step-N-Hold	50 V	13:30 hours
d.	S2 Gradient	500 V	1:00 hour
e.	S3 Step-N-Hold	1000 V	1:00 hour
f.	S4 Gradient	4000 V	1:30 hour
g.	S5 Step-N-Hold	4000 V	1:00 hour
h.	S6 Gradient	8000 V	2:00 hours
i.	S7 Step-N-Hold	8000 V	4:00 hours

12. The program parameters for the 13 cm strips are:

a.	Rehydration	0:00	
b.	Running parameters	15°C	150 μA/strip
c.	S1 Step-N-Hold	50 V	13:30 hours
d.	S2 Gradient	500 V	1:00 hour
e.	S3 Step-N-Hold	1000 V	1:00 hour
f.	S4 Gradient	4000 V	1:30 hour
g.	S5 Step-N-Hold	4000 V	1:00 hour
h.	S6 Gradient	8000 V	2:00 hours
i.	S7 Step-N-Hold	8000 V	6:00 hours

13. The amount of current after beginning the program should be 3μA or less. If it is higher, it is likely the protein samples were not clean enough, and will not transfer and separate well in the Dry Strip. After 60 minutes, it should be noticeable that the dye front is migrating towards the cathode. If this has not occurred, then current is not passing through the strips and the ceramic strip holder needs to be cleaned.
14. See Running a 2-D gel SOP SP009.
15. No detergent or water should be applied to the contact plate surface.

References:

Berkelman, T., T. Stenstedt. 1998. 2-D Electrophoresis Using Immobilized pH Gradients: Principles and Methods. Amersham Pharmacia, SE-751 84 Uppsala, Sweden.

"2-D Gel Procedure using the IPGphor IEF System," Benjamin J. Espinosa, Mycobaterial Research Laboratories, CSU, Fort Collins CO.