First Dimension Isoelectric Focusing of 18-cm IPG Strips Using
the Multiphor II System
AfCS Procedure Protocol PP00000147
Version 1, 04/11/03

This protocol describes isoelectric focusing (IEF) using the Multiphor II System. This method of focusing is conducted at high voltages and low currents due to low ionic strength within the strips. During IEF, the current decreases while the voltage increases. A low initial voltage minimizes sample aggregation and allows the parallel separation of samples with differing salt concentrations. The program used is dependent on the size of the strip being focused and the quantity of protein on the strip.

Preparation for Isoelectric Focusing
1. Clean all components of the Immobiline DryStrip Kit using a mild detergent. Ensure all components are dry.
2. Connect red bridging cable (confirm electrical connections).
3. Set the MultiTemp III Thermostatic Circulator cooling unit to 20 °C, at least 30 min before use.
4. Make sure the cooling plate is level.
5. Pipette 10 ml PlusOne DryStrip Cover Fluid directly onto cooling plate.
6. Place DryStrip Reswelling Tray on cooling plate with anodic (red) electrode at the top. Remove any large air bubbles from underneath the plate; this ensures good thermal contact.
7. Connect anode (red) and cathode (black) electrode strip tray leads to the unit.
8. Pipette 15 ml cover fluid onto DryStrip tray.
9. Place DryStrip aligner (groove side up) onto the surface of the DryStrip tray. Aligner may be numbered 1 through 13 for easier strip placement.
10. Cut two IEF electrode contact strips, 11 cm each. Moisten each strip with 0.5 ml purified water and blot with filter paper. The strips should be damp; too much moisture within the strips will cause uneven focusing of the sample. Set aside.
11. Remove rehydrated 18-cm immobilized pH gradient (IPG) strips from strip holder/reswelling tray. Rinse with purified water and blot back of strip dry.
12. Place individual rehydrated IPG strips (acidic/pointed end up) into wells of the DryStrip aligner. The strips should be aligned and placed next to each other in the center lanes of the tray.
13. Place moistened electrode contact strips across the cathodic and anodic ends of the strips. Ensure contact strips make contact with the gel surface of each strip and that there is no gel surface visible behind the contact strips.
14. Position each DryStrip Kit electrode at its respective end over each contact strip (anode=red/top; cathode=black/bottom). Make sure the red=right/black=left ends correspond to the side of the tray providing electrical contact.
15. Press down, ensuring each IPG strip is still aligned in its groove and the electrode/contact strips are straight across the IPG strips.
16. Fill tray/aligner with 100 ml cover fluid. The strips should be covered to ensure effective focusing (prevents dehydration of strips).
17. Attach lid to the base, making sure the electrode leads are firmly in place.
18. Turn on power supply and begin the preset focusing program.
19. After program has run, place the focused strips into chilled, labeled glass tubes and freeze at -80 °C until equilibration and second-dimension electrophoresis can be performed.

IEF Program
The Amersham protocol based on volt hours is the focusing program used for optimal results.

Gradient Program (~20 hr focusing time)

<table>
<thead>
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<th>Step</th>
<th>Voltage (V)</th>
<th>Current (mA)</th>
<th>Power (W)</th>
<th>V hrs</th>
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<td>1</td>
<td>5</td>
<td>10</td>
</tr>
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<tr>
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<tr>
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<td>1</td>
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<tr>
<td>5*</td>
<td>500</td>
<td>1</td>
<td>5</td>
<td>17,000</td>
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</table>

*Optional (holding) step

Reagents and Materials
Multiphor II System: Amersham Biosciences; catalog no. 18-1018-06

Multiphor II Accessories Immobiline DryStrip Kit: Amersham Biosciences; catalog no. 18-1004-30, includes
- DryStrip aligner; catalog no. 18-1004-34
- IEF electrode strip; catalog no.18-1004-40
- Electrode, anode; catalog no. 18-1018-66
- Electrode, cathode; catalog no. 18-1018-67
- Tray and electrode holder; catalog no. 18-1004-31

MultiTemp III Thermostatic Circulator: Amersham Biosciences; catalog no. 18-1106-33

PlusOne DryStrip Cover Fluid: Amersham Biosciences; catalog no. 17-1335-01

Immobiline DryStrip Reswelling Tray: Amersham Biosciences; catalog no. 80-6371-84

EPS 1001 Power Supply: Amersham Biosciences; catalog no. 18-1130-03

IPGphor Isoelectric Focusing Unit: Amersham Biosciences; catalog no. 80-6414-02

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