Focused immobilized pH gradient (IPG) strips must be equilibrated with sodium dodecyl sulfate (SDS) buffer prior to second dimension electrophoresis. The equilibration procedure consists of two steps. The first step contains dithiothreitol (DTT), which reduces denatured proteins. The second step contains iodoacetamide, which covalently modifies the reduced sulfhydryls.

1. Prepare 2-D DTT equilibration solution (10 ml per 18-cm strip).
2. Prepare 2-D iodoacetamide equilibration solution (10 ml per 18-cm strip).
3. Remove the glass tubes containing IPG strips from -80 °C freezer and allow to warm to room temperature for 10 min.
4. Add 10 ml 2-D DTT equilibration solution to each glass tube. Make sure gel side of strip is facing up and overlaid with solution. Place on a rocker for 10 min. Carefully pour solution out of tubes.
5. Add 10 ml of 2-D iodoacetamide equilibration solution to each glass tube. Make sure gel side of strip is facing up and overlaid with solution. Place on a rocker for 10 min. Carefully pour solution out of tubes.
6. Strips are now ready for second dimension electrophoresis.

**Reagents and Materials**

- Immobiline DryStrip, pH 4-7, 18 cm: Amersham Biosciences; catalog no. 17-1233-01
- 2-D Dithiothreitol equilibration solution (2-D DTT equilibration solution); AfCS Solution Protocol PS00000489
- 2-D Iodoacetamide equilibration solution; AfCS Solution Protocol PS00000490
- Glass tubes: Fisher Scientific; catalog no. 14-957-76F
- Platform rocker: Fisher Scientific; catalog no. 05-450-34

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