SOP: SP009

Running of Two Dimensional Gels

Materials and Reagents:

1. IPG Dry strip with focused protein (from SOP SP008)
2. Rehydration buffer, 2 X 20ml (note 1)
3. DTT
4. Iodoacetamide
5. Bromophenol blue
6. 2% agarose
7. 2D SDS PAGE Gel (see SOP SP006)
8. 10X Running Buffer (see SOP R008)
9. Pan sonicator
10. Forceps
11. Equilibration tray
12. Filter paper
13. P1000 pipetman
14. P1000 pipet tips
15. Polyacrylamide Gel Running Apparatus (see SOP SP007)

Protocol:

1. _____ Prepare 2 X 20ml of the Equilibration Buffer (note 1).

2. _____ Add 100mg DTT to one of the 20ml Equilibration Buffer aliquots for Equilibration Solution #1.

3. _____ Pan sonicate Equilibration buffer until DTT is completely in solution.

4. _____ Use forceps to remove IPG Dry Strip with focused protein from the electrophoresis tray and place in a equilibration tray with 15ml Equilibration Solution #1. Cover and place on a platform shaker for 10 minutes.

5. _____ Add 0.9g iodoacetamide and a few grains bromphenol blue to 20ml Equilibration Buffer for Equilibration Solution #2.

6. _____ Pan sonicate Equilibration buffer until iodoacetamide is completely in solution.

7. _____ Discard Equilibration Solution #1 and add 15ml Equilibration Solution #2 to the IPG Dry Strip. Cover and place on a platform shaker for 10 minutes.

8. _____ Melt 2% (w/v) agarose in the microwave and mix a solution of 1 part 2% agarose to 2 parts Equilibration solution #2 (you will want approximately 1ml of this mix).

9. _____ Use forceps to remove the IPG strip from the buffer and touch the strip to a dampened piece of filter paper to remove any excess buffer.

10. _____ Set up a 2D PAGE Gel (see SOP SP007). Fill the chamber with running buffer.

11. _____ Place the IPG strip into the 2D well of the 2D PAGE Gel with the gel facing out and the basic side of the IPG strip to the left. Gently push down on the strip so it is in firm contact with the PAGE gel and there are no air bubbles between the two gels.

12. _____ Using a P1000 pipetman, overlay the IPG gel strip with the agarose/equilibration buffer and allow the agarose to solidify.

13. _____ Connect the electrodes and run the gel at 15mAMP until the dye front has almost reached the bottom of the gel.

14. _____ Remove the gel and discard the IPG gel. Stain the 2D gel with either coomassie stain (see SOP SP013) or silver stain (see SOP SP012).

Notes:

1. Equilibration buffer is made as follows (for 40 ml):

	Amount	Final concentration
1M Tris-Cl, pH 6.8	4ml	50mM
Urea	714.4 g	6M
Glycerol	12ml	30%
SDS (Sodium dodecyl sulfate)	0.4g	1%
Water, B & J	to 40 ml	

Prepare buffer just before use. 20ml is needed for Rehydration Solution #1 and another 20ml is used for Rehydration Solution #2.

References

"Two Dimensional Gel Electrophoresis". Current Protocols in Protein Science.