2D Electrophoresis
2D Cleanup kit (PSC 537)

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2D electrophoresis is very sensitive to detergents, salts and pH. This kit precipitates proteins leaving behind in solution interfering substances such as detergents (ex. SDS), salts, lipids, phenolics and nucleic acids. The kit solutions do not cause any protein changes and the protein is easily soluble at the end.

The GE 2D cleanup protocol is not included on this page (link above). However, I have included some very important changes/key points to the protocol. Each note has the Step # in the GE protocol. Please read them through carefully before starting.

Besides cleaning up your sample, you are also concentrating the sample. **Goal: high concentration and low volume.**

Notes: • Always wear nitrile gloves when handling everything from sample prep until loaded on MALDI plate for analysis.

Protocol changes/key points

1. 2D Quant kit or other quantification method before doing Cleanup. You need to know how much protein you have so you don't do too much. You should only do 100 ug protein/tube.

2. Keep Wash buffer at –20oC. This way you won't forget to chill it before you use it.

3. After EVERY addition of a solution, vortex vigorously for 20 sec. This can get very tedious, but you will get improved results with diligent vortexing.

4. Do replicates – 100 ul/sample X 2. You should not go over 100 ug, but unless you do the 2D Quant kit before Cleanup, you won't know how much protein you have.

5. Step 2. After vortexing 20 sec, on ice for 20 min.

6. Step 3/4. Add coppt. and vortex 20 sec.. Place tube in microfuge with cap hinge facing outward. Spin 14,000 x g (max for microfuges) for 5 min. either at RT or cold.

7. Step 5. Working 1 tube at a time (don't remove all the tubes and then remove supernatant), remove supernatant very carefully. You do not want to disturb the pellet. Run tip down side of tube away from the pellet and remove liquid.

8. Step 6. Read protocol carefully. It is very important to reposition tube in microfuge

the same way all the time. This is so that you do not disturb the pellet. Brief pulse – 30 sec. Remove last of liquid.

9. Step 7. Layer co-precititant very carefully , drop-by-drop on top of the pellet. Let sit on ice 5 min. with tube slanted. 10. Step 9. Pellet does not disperse in H2O in 5 – 10 sec. It takes much longer that

10 sec. Remember, you are not resuspending the pellet, just breaking it up.

11. Step 11. After adding the wash buffer and wash additive, you can leave the tubes at – 20oC o/n.

12. Step 12. Spin 5min. Handle pellet the same as #5 and 6 above.

13. Step 13. Allow the pellet to dry a couple of minutes. Don't dry too far – difficult to resuspend. Too far – the pellet looks powdery. If it does go too far, just add some wash buffer and repeat #5 and #6 above.

14. Step 14. Resuspend pellet in 50 ul rehydration buffer total (started with 2 - 100 ul tubes); 25 ul/ tube . Goal: high concentration and low volume. You want 1/8 – 1/4 of the IEF volume to be sample + 2X sample buffer. The remaining volume (depends on length of strip) is the rehydration buffer.

15. Step 14. Vortex well. Combine replicates. Spin down foam in tube to get last drops of protein.