# 2D Electrophoresis
# 2D Quant kit (PSC 537)

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Proteins for 2D electrophoresis require accurate quantification. The correct amount of protein must be loaded for whatever detection system you are using. Also, if you are comparing spots in 2 gels, the amount of protein loaded must be the same to have valid analysis. The problem with most quantification protocols is that they are not compatible with 2D solutions used to solubilize the proteins. This kit precipitates the proteins while leaving interfering substances in solution. Precipitated proteins are resuspended in a copper containing solution. The copper binds to the protein and the unbound copper is measured with a colorimetric agent. The color density is inversely related to the protein concentration. The protocol is compatible with 2%SDS, 1% DTT, 8 M urea, 2M thiourea, 4% CHAPS, 2% Pharmalyte and 2% IPG buffer.

The GE 2D Quant 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. All steps are done at RT. I have an Excel worksheet template for calculating the standard curve and sample concentrations. Send me an e- mail (dwalthall@gsu.edu) and I will send it to you.

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

**Protocol changes/key points**

1. Preliminary Prep. Prepare fresh each time. Ex. 34 tubes; 1 ml of color reagent/tube. Combine 34 ml of Color reagent A with 340 ul of Color reagent B.

2. Step 1 and Step 2. Do duplicates for standard and samples. After pipetting the correct amount, wipe the outside of the tip with a Kimwipe before putting it into the tube.

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. Step 3. Get all of liquid out of pipet tip. After you dispense the solution, let the liquid settle at the end of the tip and dispense again.

5. Step 5. Spin 7 – 10 min.

6. Step 6/7. The pellet is very fragile and spread over the side of the tube. Working 1 tube at a time, remove supernatant very carefully. Don't remove all the tubes and then

remove supernatant. You do not want to disturb the pellet. Run tip down side of tube and remove liquid. 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. It is extremely important that you remove ALL of the liquid.

7. Step 8. Order in which the H2O and copper are added to the tubes does not matter.

8. Step 9. Working color reagent prepared in #1 above – pipet into tube quickly one at at time and invert to mix immediately.

9. DIGE procedure. Repeats steps 3 -5. Shelby never uses this. The standard procedure works fine for her samples.