Protein Extraction from Brain Tissue, Julie Miller, updated 2011

1) For tissue, the amount of Ripa lysis buffer to be used depends on the size of your tissue. If your tissue is the size of a rat/bird brain, then 1/3 of this brain should be homogenized in 200-300ul, if it is more the size of bird nucleus Area X (1mm in diameter, small round circle) then probably 40ul of lysis buffer is sufficient for bilateral tissue punches. You want 10ul of Sigma Protease inhibitor cocktail (to prevent protein degradation) for every 1000ul of Ripa lysis buffer.

2) After homogenizing, keep on ice while assaying, otherwise, stick the tube at -80°C until you will assay it and store it in more permanent buffer (loading sample buffer with beta-mercaptoethanol). At -80°C, the tissue will be fine for a week or so-after that, you will get degradation unless you store it in betamercaptoethanol. Minimize your freeze/thaws.

3) Perform the Bio-Rad RC DC protein assay:
   Preparation: Protein samples should be kept on ice. Tubes for the protein assay can be kept at room temperature. Make up Bovine Standard Albumin (BSA) dilutions - make up in lysis buffer (protease inhibitors not needed) and store at 4°C-good for 1 month. Use BSA dilutions of 0.2mg, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, and 1.0mg/ml. Use fresh tubes and pipet 25ul of each dilution in a fresh tube for the assay. You should also prepare a ‘blank’ tube of 25ul with lysis buffer only, for ‘blanking’ on the spectrophotometer. Make up Reagent A’ by adding 5ul of Reagent ‘S’ (little bottle) to 250ul of Reagent ‘A’ (blue bottle): generally, I make up 100ul of ‘S’ + 5ml of ‘A’ as each sample will require 127ul of this A’ Reagent. Vortex this in a conical tube. I make this A’ stock up fresh every assay.

4) For protein samples: For Area X bilateral punches, the tissue is the size of 1mm, so for the assay, I would make up 1 tube with 3ul of your sample + 22ul of lysis buffer (for 25ul total). For cerebellum whole tissue or mouse olfactory bulb, try 1ul or 1ul of a 1:5 dilution for your assay. If your absorbance reading is below the standard BSA curve, you will need to repeat the assay using >ul of your sample.

5) Add 125ul of RC Reagent I into each tube, vortex, and let the tubes sit at room temp for 1 min.

6) Add 125ul of RC Reagent II into each tube, vortex, then centrifugue the tubes at 15,000g for 4 minutes at room temperature using the A-15 rotor on large centrifuge or benchtop centrifuge.

7) Look for the small pellet on the side or bottom of the tube then discard supernatant by inverting the tubes on diapers. Use a pipet tip to carefully remove all liquid without disturbing the pellet.

8) Turn on spectrophotometer to allow for calibration. Add 127ul of Reagent A’ to each tube and vortex then incubate tubes at room temperature for 5 minutes or until pellet dissolves. Vortex before proceeding to next step.

9) Add 1ml of DC Reagent B (big brown bottle) to each tube, vortexing immediately. Wait 15 minutes. Then, use spectrophotometer – select Lowry assay at 750nm then program in your BSA standards. (5 standards). Use plastic cuvettes – pour each sample into cuvette. You will need to use the blank 2x- once to blank before running the standard and then a second time to blank before measuring your protein samples.

Plot in excel- BSA standards on x-axis and their absorbances on y-axis. Add a best fit line by going to “Chart” then “Add trendline” then ‘Options’ Select ‘Display Equation on Chart’ and ‘R squared value.’ You want a line close to $R^2 >0.95$.

Use the $y=mx+b$ equation for the slope of the line to calculate your protein concentration:
y=absorbance value and solve for x
x=protein sample

So x=number x 25/sample volume used for assay = ug/ul of protein concentration

Example: absorbance for a cell culture =0.127
y=0.1205-0.0059
solve for x
x=0.572 x 25/4ul used for assay = 3.58 ug/ul total protein (make sure you use 0.2, 0.4 mg/ml for your BSA curve in excel)

Take your original stocks sitting on ice and flick tube to mix. Do aliquoting under hood b/c of toxic betamercaptoethanol. If you want to make 50ug aliquots in 2x loading sample buffer (1ml= 950ul of Laemmli dye 2x +50ul of beta mercaptoethanol) then you would take=
13.97ul of protein + 11ul of 1xPBS buffer + 25ul of Loading sample buffer. Store at -80°C.