

## Isopropanol/Hexane Brain Tissue Protocol

Biomarker Applications and Chemistry Development Group

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### Materials

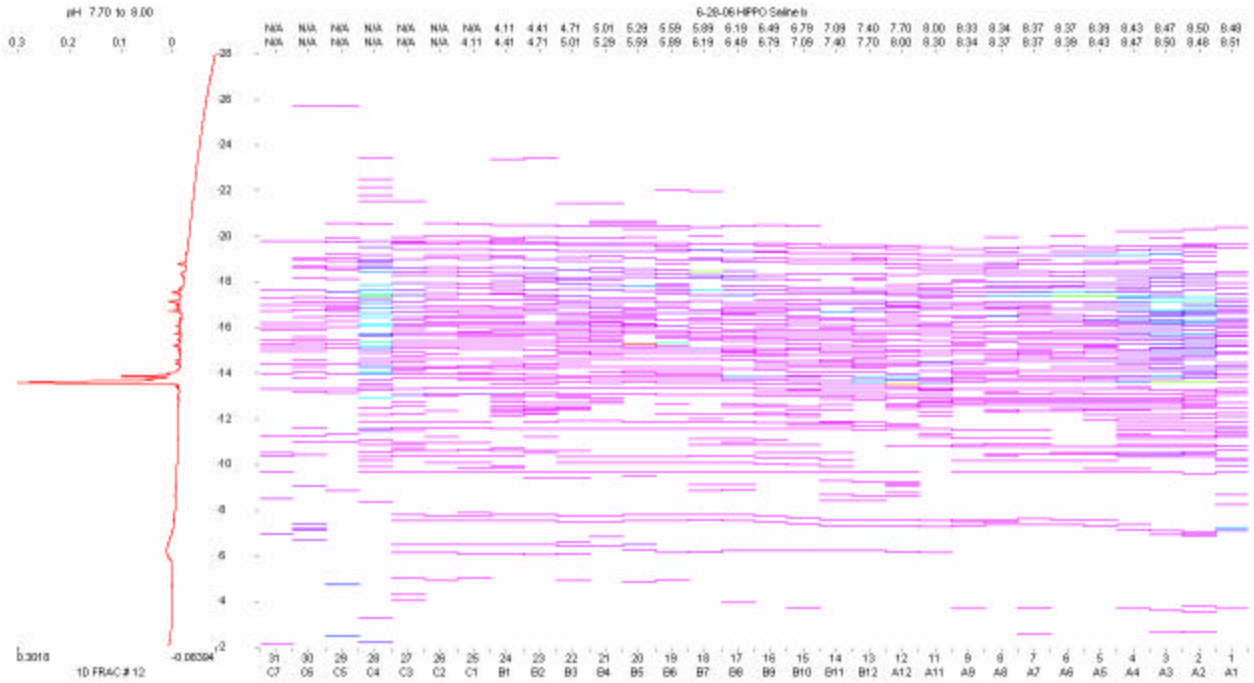
1. Tapered tissue homogenizer, 5 mL with Fluoropolymer Resin pestle (Wheaton, part number 358141)
2. 50 mL Start Buffer
3. One vial of General Lysis-Denaturing Buffer **NOT** containing TCEP
4. One PD 10 Column
5. 2 mL HPLC grade water
6. Two 15 mL polypropylene centrifuge tube (VWR, catalog number 20171-024)
7. Four 2 mL polypropylene micro-centrifuge tubes capable of withstanding 20,000 xg (Axygen, part number MCT-200-C)
8. Two 5  $\mu$ m spin filters (Millipore, part number UFC4 0SV 25)
9. Two 0.45  $\mu$ m spin filters (Millipore, part number UFC4 0HV 25)
10. ProteomeLab SP with supplied rotors. The rotor models used in this protocol are specified below in parenthesis after the rotor type. If not available, need a refrigerated centrifuge capable of centrifuging 2 mL polypropylene micro-centrifuge tubes at 20,000 xg, 50 mL polypropylene centrifuge tubes at 8000 xg and 4 mL tubes at 3900 xg (using a swinging bucket rotor).
11. One 20-200  $\mu$ L variable microliter pipettor, e.g., Pipetman P200, with appropriate pipet tips.
12. One 200-1000  $\mu$ L variable microliter pipettor, e.g., Pipetman P1000, with appropriate pipet tips.
13. Several disposable transfer pipets; 4 mL (VWR catalog number 16001-178)

### Methods

1. Remove tissue from the -80°C freezer and weigh. Record the weight of the tissue.
2. Remove a vial of General Lysis-Denaturing Buffer from the freezer and place in a water bath at 37°C for a couple of minutes to thaw. Gently rotate vial in a back and forth motion to mix the buffer. Then add 2.0 mL General Lysis-Denaturing Buffer **NOT** containing TCEP in a 5 mL homogenizer. Add 0.5 mL HPLC grade water to increase the volume to 2.5 mL.
3. Homogenize with the Teflon pestle at room temperature until the sample is completely homogeneous (about 20 strokes).
4. Let the homogenate stand for 30 min at room temperature to insure proper denaturation of the proteins.
5. Divide the sample into two aliquots into 2.0 ml polypropylene centrifuge tubes and centrifuge at 20,000 xg for 60 min at 18°C in the F2402H rotor.
6. Combine the supernatants into a single 15 mL polypropylene centrifuge tube. Assume the volume is 2.5 mL.

7. Add 5 mL of isopropanol and 7.5 mL of hexane to the supernatant. Cap the 50 mL tube and shake by hand for 5 seconds.
8. Place in the centrifuge for 30 min at 10°C. This allows the protein to precipitate.
9. Centrifuge the sample at 8000  $xg$  for 15 min at 10°C using the fixed angle rotor F0685.
10. Gently remove the supernatant from the tube using a disposable plastic pipet. Do not let the pellet dry out.
11. Add 12.5 mL 100% methanol to the pellet, which will remove phospholipids trapped in the pellet. Vortex for 30 sec.
12. Centrifuge the sample at 8000  $xg$  for 15 min at 10°C using the fixed angle rotor F0685.
13. Remove a vial of General Lysis-Denaturing Buffer from the freezer and place in a water bath at 37°C for a couple of minutes to thaw. Gently rotate the vial in a back and forth motion to mix the buffer. Add 2.0 mL General Lysis-Denaturing Buffer to the 50 mL homogenizer. Carefully remove the pellet using a flat spatula and place in the General Lysis-Denaturing Buffer. Add 0.5 mL of HPLC grade water to the 50 mL conical polypropylene tube to remove any of the pellet remaining in the tube. Using the 1000  $\mu$ L pipetman, transfer the 0.5 mL to the sample in the homogenizer. Homogenize the sample in the buffer until completely homogeneous (about 5 strokes). Allow to sit at room temperature for 30 min.
14. Divide the sample into two aliquots into 2.0 mL polypropylene centrifuge tubes and centrifuge at 20,000  $xg$  for 15 minutes at 18°C in the F2402H rotor.
15. Bring the volume up to 2.5 mL in a polypropylene centrifuge tube (15 mL) using Start Buffer then add the sample to a PD10 column previously equilibrated with Start Buffer.
16. Add 3.5 ml of Start Buffer and collect the eluted protein.
17. Wash two 5  $\mu$ m and two 0.45  $\mu$ m spin filters with 2.0 mL Start Buffer and centrifuge at 3901  $xg$  in a swinging bucket rotor (SX4250) at 18°C. This will require 1 minute for the sample to completely filter.
18. Add the sample to two 5  $\mu$ m spin filters and centrifuge at 3901  $xg$  in a swinging bucket rotor (SX4250) for 1 minute at 18°C.
19. Add the sample to two 0.45  $\mu$ m spin filters and centrifuge at 3901  $xg$  in a swinging bucket rotor (SX4250) for 1 minute at 18°C.
20. Determine the protein content of the sample using the microBCA and A280 protein assays.
21. Inject 4 mg of protein on the HPCF column. Bring the volume of sample up to 5 mL using Start Buffer.

The result for a typical fractionation is shown below.



pH/Hydrophobicity map of rat hippocampal brain tissue (4 mg) fractionated on the ProteomeLab PF 2D. Each first-dimension pH fraction is shown as a lane numbered from 1-31, with the pH limits of each fraction at the top of each lane. The second-dimension runs of each first-dimension fraction are displayed in each lane, with increasing retention time from bottom to top. The panel on the far left is the chromatogram for fraction 12.