

Protocol for labeling with d0/d6-GABA photo-beads

Updated 7/29/03

Keep light-sensitive beads out of direct light as much as possible.

1. Digest sample proteins in 100 μ l suitable buffer (pH 8) with 2-4 μ g trypsin, overnight at 37°C.
2. Reduce each digest in 5 mM TCEP, 30 min. at room temp.
3. Measure 5 mg each of d0- and d6-GABA beads into foil-covered or dark tubes (brown ICAT tube with 1 ml glass vial as liner is perfect).
4. **Label:** Add reduced digests to beads, shaking immediately. Shake 30 min.
5. Quench labeling reaction with 100 μ l water plus 2 μ l β Me.
6. **Wash:** Combine beads by loading onto foil-wrapped Bio-Spin column (732-6204), washing with water and MeOH to transfer all beads. (Retain flow-through of each labeling reaction separately for analysis of non-cysteine-containing peptides.) Wash with:
 - a. 6 x 1 ml 1.5 M NaCl
 - b. 6 x 1 ml 0.1% TFA
 - c. 6 x 1 ml 80% ACN/0.1% TFA
 - d. 3 x 1 ml MeOH
 - e. 6 x 1 ml $\text{NH}_4\text{OH}:\text{MeOH}$ (1:9)
 - f. 6 x 1 ml MeOH
 - g. 6 x 1 ml water(The number of these steps might be reduced if someone has the time to test them.)
7. Seal bottom of Bio-Spin with parafilm and cap. Suspend beads in 100 μ l 20 mM Tris, pH 8, and 2 μ l β Me.
8. **Photo-cleave:** Expose beads to UV light, 365 nm, 50 watts, for 20 minutes, shaking continuously.
9. **Wash:** Wash from Bio-Spin with 3 x 100 μ l 80% ACN/0.4% acetic acid. Spin and transfer to another tube if glass beads leaked through spin column.
10. Remove ACN in Speed-Vac, about 15 min., yielding volume of about 160 μ l. Check that pH is acidic.
11. **Capture:** Load sample onto microcapillary SCX column (~200 μ m x ~3 cm) at 5-10 μ l/min.
12. Wash with 1 x 100 μ l 0.4% acetic acid at 5-10 μ l/min.
13. Wash with 1 x 100 μ l 80% ACN/0.4% acetic acid at 5-10 μ l/min.
14. **Elute by salt steps:** Wash with 2 x 100 μ l 25% ACN/5 mM phosphoric acid.
15. Elute in 50-75 μ l each 60, 120, 180, 300, and 600 mM KCl.
16. Wash with 50 μ l 25% ACN/5 mM phosphoric acid (to reduce salt before base step).
17. Elute in 50-75 μ l $\text{NH}_4\text{OH}:\text{MeOH}$ (1:9).
18. Dry salt fractions to 10 μ l.
19. Dry basic eluate completely in Speed-Vac and suspend in 10 μ l water or 0.4% acetic acid.

SCX column:

description	manufacturer	catalog #	quantity
Frit-in-a-Ferrule	Upchurch	P-274X	10-pack
Super Flangeless Nuts	Upchurch	P-246X	10-pack
PFA Teflon tubing, 0.50 mm x 5'	Upchurch	1620	1
PFA Teflon tubing, 0.75 mm x 5'	Upchurch	1630	1
Polysulfoethyl A, 5 μ m, 300 A	PolyLC	BMSE0503	1 gm.
Gastight syringe, 100 μ l, RN 22s/2"/3	Hamilton	81065	1
Replacement needles, 22s/2"/3	Hamilton	0160823	3-pack