Materials List for:

Comprehensive Profiling of Dopamine Regulation in Substantia Nigra and Ventral Tegmental Area

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Materials

Name	Company	Catalog Number	Comments
HPLC system:			
The basic system consists of a Shimadzu LC10-ADvp HPLC pump, a Waters WISP 717 automatic sample injector, a 250 X 4.4 mm 5 micron Spherosorb ODS-**1** C18 reverse-phase column (Waters), a Bioanalytical Systems (BAS) TL12 dual glassy carbon electrode, two BAS LC4B electrochemical detectors, and a Waters Empower 2 data collection and integration system.			
The column is maintained at 30-45 °C (BAS LC22A column heater). The mobile phase is 0.1 M sodium phosphate (pH 3.0), 0.1 mM EDTA, 0.2-0.4 mM 1-Octane Sulfonic Acid (Eastman-Kodak), and 0.35% acetonitrile (v/v), filtered through a 0.45 micron filter. Flow rate is of 1.2 ml/min. Four liter batches of mobile phase are optimized for separations by adjusting the pH, Octane Sulfonic Acid and column temperature. The mobile phase is recycled, and is continuously purged with helium gas to remove dissolved oxygen. Recycling of the mobile phase is almost essential to maintain good resolution for a reasonable period of time. The mobile phase shelf-life is maintained by using a flow switch (controlled by the integrator) to divert to waste the first 2-7 min of each run.			
The electrodes are maintained at potentials of approximately 0.78 and 0.95V with respect to a Ag/AgC1 reference electrode. The electrode at the higher potential is used exclusively for the determination of tryptophan (and the NMDA internal standard). The 0.78 V potential provides a superior signal to noise ratio for detection of the monoamines and compounds, other than tryptophan. The chromatograms are stored on the hard drive of the Empower workstation, and subsequently processed and the data transferred directly into an Excel spreadsheet for computation of metabolite amounts and compilation of group data.			
Pump: Shimadzu LC-10AD Cell: BAS Cross Flow. Glassy carbon working electrode at 0.780 and 0.950 V potential. Detector: BAS LC-4B operated in dual channel mode. Data Acq. System: Waters Empower Pro 2. Injector: Waters WISP 717 Column: Waters Spherosorb ODS-1, 5 µM particle, 4.4 mm X 250 mm.			
Sodium Dodecyl Sulfate (SDS)	J.T. Baker	4095-02	
Trizma Base	Sigma	T1503-1KG	
Trizma HCl	Sigma	T3253-1KG	
Glycerol	Sigma	G8773-500 mL	
PVP-40	Sigma	PVP40-1KG	
dPBS	Gibco	21600-069	
Tween20	Sigma	P1379-500 mL	
Glycine	Sigma	G8898-1KG	
Ponceau S	Fluka	81460	
Bromophenol Blue	Sigma	B8026-5G	
Dithiothreitol	Sigma	D-9163	
Protein Standard 2 mg BSA	Sigma	P5619-25VL	
Pierce BCA Protein Assay Reagent A	Thermo-Fisher Scientific	23223	
Precision Plus Protein Standard	Bio Rad	161-0373	
[125I]-protein A, specific activity	Perkin-Elmer		

Table 2. Specific reagents.

10% SDS			10 g SDS, 100 mL DI H_20
1% SDS (pH to 8.2)			10 mL 10%SDS 60.5 mg Trizma Base 37.22 mg EDTA 90 mL DI H_20
Copper II Sulfate Solution			1 g Copper II sulfate 25 mL DI H_20
3X Sample Buffer			Trizma Base 2.27 g SDS 6 g Dithiothreitol 0.463 g Glycerol 30 g Bromophenol Blue 10 mg D I H_20 (initially add above reagents to 40 mL of H_20 in a graduated cylinder; then add H_20 until volume reaches 100 mL) HCl (add as needed to reach pH of 6.85) Freeze solution in 50 2.0 mL tubes. (makes 100 mL): Volume of 3X Sample Buffer needed = ½ volume of SDS used in sample.
1X Sample Buffer			Dilute 3X Sample Buffer down to 1X sample buffer using DI H_20
10X Running Buffer (Makes 4 L)			Trizma Base 121.1 g Glycine 577 g SDS 40 g
10X Transfer Buffer: (Makes 4 L)			Glycine 360 g Trizma Base 96 g
Ponceau			Ponceau S. 0.5 g Acetic Acid 5 mL DI H_20 95 mL
.2% HCl Solution			5.2 mL HCl in 500mL of DI H_20
PVP-T20 Blocking Soln. (Makes 4 L)			PVP-40 40 g dPBS 38.2 g Tween20 2 g Thimerisol 0.4 g 1M Tris pH 7.6 (60.6 g Tris HCl + 13.9 g Tris Base in 500 mL DI H_20)- 50 mL
10X Blot Buffer (Makes 4 L)			Tween 20 20 g Tris Base 14 g Tris HCl 61 g

Table 3. Protein Assay and Western Blotting Formulas.

Tyrosine hydroxylase standards: The calibrated TH protein and phosphorylation standards used by this laboratory are derived from PC12 cell extracts, which were analyzed for TH protein content and phosphorylation stoichiometries against a previously calibrated TH standards that ultimately originated from the laboratory of Dr. John Haycock [11].