HPLC ANALYSIS OF ADP-RIBOSYL CYCLASE (NEURAL MICE MEMBRANES)

1. *Hank's Balanced Salt Solution*

2. *HEPES Buffer*
   -10mM HEPES, 148mM NaCl, 5mM KCl, 1.8mM $CaCl_2$, 0.3mM $MgCl_2$, 5.5mM Glucose; pH 7.0
   -for 100mL solution: 238mg HEPES, 864.9mg NaCl, 37.275mg KCl, 26.465mg $CaCl_2$, 6.1mg $MgCl_2$, 99.11mg Glucose; adjust pH with KOH and HCl

3. *Solvent A*
   -95% H_2O, 5% Methanol, 0.15M NH_4
   -for 1L solution: 950mL H_2O, 50mL Methanol, 11.562g NH_4AC

4. *Solvent B*
   -50% H_2O, 50% Methanol, 0.15M NH_4
   -for 1L solution: 500mL H_2O, 500mL Methanol, 11.562 NH_4AC

1. Immolate mouse via cervical dislocation followed by rapid decapitation.
2. Dissect cerebral hemispheres from neonatal mice.
3. Remove meninges in HBSS.
4. Triturate cerebral hemispheres into homogenous solution using plastic pipette.
5. Centrifuge 3 minutes at 4000 RPM (until pellet forms).
6. Remove HBSS.
7. Create microsomes:
   a. Homogenize neural cells with glass homogenizer in HEPES Buffer.
   b. Centrifuge 10,000g for 30 minutes (pellet is nuclear and granular fraction).
   c. Collect supernatant.
   d. Centrifuge for 100,000g for 90 minutes (membrane and cytosolic fraction).
   e. Collect supernatant.
   f. Redisolve pellet in 80uL of HEPES Buffer.
   g. Set some solution aside for protein assay, and, if necessary, aliquot the rest storing in liquid nitrogen.
10. Protein concentration assay with microsomes in HEPES Buffer previously set aside.
11. Incubate the supernatant from 100ug of protein(cells) with a final concentration of lmM NGD at 37°C for 2 hours.
12. Centrifuge the reaction mixture through Amicaon microultrafilters at 4°C at 13,800g for 15 minutes to remove proteins.
13. Use cGDP-R on computer detection method to detect cGDP-R production. Standards:1uM cGDP-R
14. Take 100uL/vial for HPLC analysis.

1. Column type: 55852-03 150mm x 4.6mm, 3um
2. Fluorescence Detection: Excitation: 300nm
   Emission: 410nm
3. Flow rate: 0.8 ml/minute
4. Runing buffer (adjust pH to 5.5 with acetic acid):

Solvent A.	H_2O	950mL/L
	5% Methanol	50mL/L
	0.15M NH_4AC	11.562g/L
Solvent B.	H_2O	500mL/L
	5% Methanol	500mL/L
	0.15M NH_4AC	11.562g/L

5. Timetable:

0.01	Solvent A: 100%	Solvent B: 0%
4.00	Solvent A: 100%	Solvent B: 0%
12.0	Solvent A: 75%	Solvent B: 25%
16.0	Solvent A: 0%	Solvent B: 100%
20.0	Solvent A: 0%	Solvent B: 100%
25.0	Solvent A: 100%	Solvent B: 0%

HPLC Cleaning Procedure

1. Check Filter (PTFE)
   a. Open Valve
   b. 100% water
   c. set flow rate to 5.00 ml/minute
   d. if pressure is less than 10 bar ⇒OK
   e. if pressure is greater than 10 bar ⇒ change

2. Guard Column Cleaning
   a. Remove guard column (front end first)
   b. Come back to 1AB. Remove gray foal pad
      i. Look for cleaning stuff
   c. With the wire, clean all of the white foam from the middle of the column, putting clean pieces in water in beaker
   d. Sonicate for 30 seconds, 3 times with water
   e. Sonicate for 30 seconds, 3 times with Ethanol
   f. Dry pieces with forced air in the hood
   g. Put together first on sides where arrow points ⇒ put new filters in

h. Funnel powder in with arrow pointing down
    i. Pack very tightly
i. Put column back together
    i. Align arrow with the flow
    ii. Put the backside on first and secure it, do NOT put front side on yet
    iii. Drop flow rate to 0.8, close purge valve
    iv. Wait until there is a consistent flow from the column
    v. Once the constant drip begins, open valve and replace front end
j. Close valve ⇒ leak sensors
    i. Check for leaks in the creases of the column by wiping using a KIM-WIP
    ii. If leaking, tighten or check to see if assembled correctly

HPLC Sampling Protocol

1. Before start:
    a. Light Bulb warm up (~ 2 minuites)
    b. Method is on ERYN-UV.M
    c. Buffer solution volume is the actual amount
    d. Sequence parameters
        i. Change date
        ii. Ensure that post-sequence CMP is checked
    e. Sequence tables (under ERYN-UV.M)
        i. Enter vials and quantity
        ii. Injection rate is 20 ul
        iii. Sample amount is 100 ul
        iv. Last sample set to WASH-2-UV
    f. Sequence output
        i. Select as specified in each method
    g. Run control tab
        i. Sample info, Change date
    h. 15 minutes before START
        i. run buffers through at 50% 'A' / 50% 'B' at the rate of 5.0 ml/ minute for 15 minutes with purge valve open
    i. Check and recheck parameters and hit START !!!!