Sample Preparation for Purine HPLC Assay

Summary

This protocol was developed to measure purines from cultured cells. Application to cultured cells was described in Lewers et al, Neuroscience, 152:761-772, 2008: Shirley et al, J Neurochem, 101, 841-853, 2007.

Sample processing

1. Sample collection
   a. Cultured cells may be harvested by scraping or trypsinization following removal of overlying culture medium. Any excess culture medium should be removed following centrifugation at 300 × g for 5 minutes.
   b. CSF samples should be collected and aliquoted.
   c. Plasma samples should be collected in EDTA coated tube and centrifuged at 1000 × g for 15 minutes at 4°C to remove blood cells.
   d. Samples may be stored indefinitely at -80ºC
2. Sample deproteinzation by perchloric acid
   a. For a single 10 cm plate of cells, the volume is about 200 µL and the sample is disrupted by probe sonication.
   b. For CSF samples, 1/10 of the sample volume of cold 1 M perchloric acid is added to sample to bring the final concentration to 0.1 M and mixed thoroughly.
   c. For plasma samples, 1/10 of the sample volume of cold 4 M perchloric acid is added to sample to bring the final concentration to 0.4 M and mixed thoroughly.
   d. The instructions on how to prepare perchloric acid solution: https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/molarity-calculator.html
3. HPLC analysis for purines on Waters instrument
   a. Centrifuge the homogenates at 10,000 × g for 10 minutes at 4ºC.
   b. Collect the supernatant into a new tube, being cautious to avoid collecting any of the pellet. Reserve the pellet for measurement of protein concentration.
   c. Adjust pH of supernatant to 7.0 by adding a small volume of 2.5% 3.5 M K_2CO_3 and store on ice for 10-15 minutes to precipitate potassium perchlorate.
   d. Centrifuge the homogenates at 10,000 × g for 10 minutes at 4ºC.
   e. Filter any remaining particulate matter from the supernatants individually by spinning in a 0.45 µM PVDF microcentrifuge filter tube at 5000 × g for 5 minutes at 4ºC.
   f. Load filtrate directly into HPLC vials for analysis on the Waters instrument.
   g. If samples are refrozen, they must be filtered through new microcentrifuge filter tubes prior to HPLC analysis.
   h. Part numbers of spin filters and HPLC loading vials:
   0.2 µm spin filters: Catalog Number: F2517-5, Thermo Scientific. National 750 µL nonsterile micro-centrifugal PVDF membrane filters (0.2 µm pore size).
   0.45 µm spin filters: Catalog Number: F2517-6, Thermo Scientific. National 750 µL nonsterile micro-centrifugal PVDF membrane filters (0.45 µm pore size).

HPLC loading vials for autosampler: Catalog Number: 186002639, Waters. Polypropylene 12 x 32 mm Screw Neck Vial, with Cap and Preslit PTFE/Silicone Septum, 300 µL Volume.

4. Protein analysis
    a. Remove all residual supernatants.
    b. Resuspend pellet in 500 µL of 2% SDS by gentle pipetting. Dissolving the pellet fully may require sonication for 10 seconds along with overnight incubation at 37ºC.
    c. Dilute 5-15 µL of sample protein in additional 2% SDS to a total volume of 50 µL for the assay.
    d. Prepare a protein standard curve that ranges from 0 to 2 mg/ml of BSA, also in 2% SDS.
    e. Add 200 µL of Pierce BCA Protein Assay reagent (mix 50 parts reagent A to 1 Part of reagent B) to each 10 µL of sample and vortex mix.
    f. Incubate 30 minutes at 37ºC.
    g. Read on the microplate spectrophotometer at 562 nm