Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Life science bldg, Lab B 310

Chemical(s)	Ascorbate, β-mercaptoethanol (BME), ethylenediamine tetraacetic acid (EDTA), Ethylene glycol, liquid nitrogen (LN), Leupeptin, PVPP, PMSF, Sodium phosphate
Process	**Soluble and Microsomal Protein extraction**
Specific Hazards	BME is flammable, and may be harmful upon skin/eye contact, ingestion or inhalation. LN is a frostbite hazard. Leupeptin – neurotoxin, Ethylene glycol – harmful when ingested, PMSF – highly toxic, PVPP – may be harmful when inhaled or ingested.
Personal protective equipment	: 3-5 mil nitrile gloves : double gloves : lab coat (except when used in microcentrifuge tubes) : chemical safety goggles (when splash potential exists)
Engineering/ventilation controls	: chemical fume hood : emergency shower and eyewash accessible
Special handling procedures and storage requirements	Store BME in the flammable cabinets under the hood in B310. Store all the other buffers on bench top.
Spill and accident procedures	Skin exposure: Rinse affected skin with plenty of water while removing contaminated clothing and shoes. Rinse for at least 15 minutes. Seek medical attention. Eye exposure: Wash eyes for at least 15 minutes, lifting the upper and lower eyelids. Seek medical attention immediately.
	Small (< 2L): Extinguish all sources of ignition. Respiratory protection is required to clean up spills. Ventilate the area. Absorb with vermiculite or spill pads and transfer to a closed container. Label and date as hazardous waste for disposal. Notify PIs.
	Large (> 2L): Evacuate the room, extinguish all sources of ignition, notify PIs and call 123 to request emergency spill assistance from Occupational Safety and Health Services.
Waste disposal	Quantities used in most protocols are so small that disposal is not an issue. Significant quantities should be collected and labeled as hazardous waste according to the SOP for Hazardous Waste Disposal.
Special approval	No special authorization needed after SOP training and reading MSDS.
Prepared by	Name/date: C-J Tsai, 8/23/2008
Reviewed by	Name/date:

Extraction of Soluble and Microsomal Proteins

Chemicals:

Ethylenediaminetetraacetic acid (EDTA)
Ethylene glycol
Leupeptin
β-mercaptoethanol (BME)
Phenylmethylsulfonyl Fluoride (PMSF)
Polyvinylpyrrolidone (PVPP)
Sodium phosphate dibasic
Sodium phosphate monobasic

Soluble protein extraction buffer

	Final conc.	Stock conc.
Sodium phosphate (pH 7.4)	100 mM	0.5 M (mix of monobasic and dibasic Na-PO4)
EDTA	10 mM	0.5 M
Ascorbate	40 mM	1.0 M
Ethylene glycol	10 % (v/v)	100 %
PVPP	5 % (w/v)	

Stir at 4°C for >10 min before use.

PMSF	1 mM	100 mM (EtOH) add before use
Leupeptin	1 µg/mL	10 mg/mL add before use
BME	14 mM	14 M add before use

Proteinase inhibitors are expensive and labile. Add before use and only the amount needed for tissue extraction.

Microsomal protein resuspension buffer

	Final conc.	Stock conc.
Sodium phosphate (pH 7.4)	100 mM	0.5 M (mix of monobasic and dibasic Na-PO4)
Ethylene glycol	30 % (v/v)	
BME	14 mM	14 M add to the final vial only

Procedures:

1. Prepare fresh extraction buffer and microsomal protein resuspension buffer, keep on ice. Aliquot 3 volumes of extraction buffer per gram of tissue to a Falcon tube and add proteinase inhibitors and BME (start with 3 g tissue and 10 mL buffer). Keep the tube on a rocker at 4°C to keep PVPP in suspension.

2. Grind tissue to a fine powder in liquid nitrogen using a pre-cooled mortar and pestle.

3. Transfer the liquid nitrogen powder to the buffer-containing Falcon tube using a liquid nitrogen- pre-cooled spatula. Mix frequently to prevent localized tissue freezing-thawing.

4. Vortex for 1 min to homogenize. Filter the homogenate through 2 layers of miracloth (prewetted with ddH_2O or extraction buffer) to a polypropylene Oakridge centrifuge tube on ice, squeeze to collect as much liquid as possible.

If you only need soluble protein:

5. Centrifuge at 10,000 g for 20 min at 4°C. Carefully remove supernatant without disturbing the pellet, and transfer to a new tube. This is crude protein extract. Make 30% (v/v) with ethylene glycol (original buffer has 10%), aliquot, snap-freeze in liquid nitrogen and store at -80°C.

If you need both soluble and microsomal proteins:
Obtain training and permission from Scott or C-J before performing ultracentrifugation.

6. After Step 4, centrifuge at 10,000 g for 20 min at 4°C. Carefully remove supernatant without disturbing the pellet, and transfer to an ultracentrifuge tube. Balance the tubes by weight to two decimal places.

7. Ultracentrifuge at 35,800 rpm for 50 min at 4°C.

8. Carefully transfer the supernatant to a new Falcon tube. This is the soluble protein fraction. Make 30% (v/v) with ethylene glycol (original buffer has 10%).

9. Resuspend the microsome pellet in 0.5-1 mL resuspension buffer. Remove 50 µL aliquot for Bradford assay (less if using the Nanodrop). Add BME to a final conc of 14 mM.

10. Determine protein concentrations. Aliquot, snap-freeze in liquid nitrogen and store at -80°C.