Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Davison Life Sciences Building, Lab B310

Chemical(s)	Sucrose, liquid nitrogen (LN), potassium chloride (KCl), sodium chloride (NaCl), glycerol, ethylenediamine tetraacetic acid (EDTA), (Ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid) EGTA, (N-2-Hydroxyethyl)piperazine-N′-2-ethanesulfonic acid) HEPES, Phenylmethylsulfonyl fluoride (PMSF), (Dithiothreitol) DTT, leupetin, Antipain and pepstain A.
Process	**Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay (EMSA)**
Specific Hazards *referred to MSDSs for more detailed information*	LN is a frostbite hazard. Leupeptin – neurotoxin, PMSF – highly toxic
Personal protective equipment	Must wear 3-5 mil nitrile gloves. Chemical safety goggles and lab coat should be worn when splash potential exist. In case of insufficient ventilation, wear suitable respiratory equipment.
Engineering/ventilation controls	chemical fume hood emergency shower and eyewash accessible
Special handling procedures and storage requirements	flammable cabinet, Rm 310 4°C refrigerator, Rm 310 store away from oxidizers store away from flammable reagents, under hood in Rm 310
Spill and accident procedures *for hazardous chemicals only*	Skin exposure: Rinse affected skin with plenty of water while removing contaminated clothing/shoes. Rinse for > 15 minutes. Eye exposure: Wash eyes for > 15 minutes. For both cases, seek medical attention immediately.
	Small (< 2L): Absorb with vermiculite or spill pads and transfer absorbed material to a closed container. Label and date as hazardous waste for disposal. Notify PIs.
	Large (> 2L): Evacuate the room, notify PIs and call 2-2200 to request emergency spill assistance from the Biosafety Office.
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 & reading MSDSs.
Reviewed by	Name/date:

Plant Nuclear Protein Extraction Protocol
(works well with *Populus* leaves, xylem, and phloem)

Solutions: (autoclave to sterilize)

NIB:	Sucrose	0.5 M	**NEB:**	HEPES (pH 7)	10 mM
	HEPES (pH 7)	10 mM		NaCl	450 mM
	EGTA	0.1 mM		EGTA	0.1 mM
	KCl	5 mM		Glycerol	5 %

Dialysis Buffer:	Glycerol	20 %
	HEPES	20 mM
	KCl	100 mM
	EDTA	0.1 mM

Protease inhibitors: DTT (0.5 mM); PMSF, Leupeptin, Antipain and Pepstain A

Procedures: (perform all the steps in the cold room or keep solutions in a ice/water bath)

1. Grind 10 g of tissues to a fine powder with liquid nitrogen (**DO NOT use sea sands**).
2. Transfer the powder to a beaker containing 100 mL NIB + 0.5 mM PMSF, 0.5 mM DTT, 2 µg/mL leupeptin, antipain, and pepstain A (precooled in an ice/water bath). Stir for 5 min.
3. Homogenize for 30 sec at medium speed using a polytron, or by vortexing at max speed.
4. Filter sequentially through 4 layers of cheesecloth, a layer of miracloth, and a 100 µ nylon mesh.
5. Spin at 2,000 xg at 4^{o}C for 10 min.
6. Wash with (<u>gently</u> resuspend in) 30 mL NIB buffer + protease inhibitors, spin as step 5.
7. Repeat step 6.
8. Resuspend in 1-3 mL of ice-cold NEB + 0.5 mM PMSF, 0.5 mM DTT and 2 µg/mL leupeptin, antipain and pepstain A. Stir for 30 min at 4^{o}C.
9. Ultracentrifuge at 35,800 rpm for 50 min at 4^{o}C.
10. Collect the supernatant into a dialysis tubing, dialyze against 700 mL cold dialysis buffer + 0.5 mM PMSF, 0.5 mM DTT, 2 µg/mL leupeptin, antipain, and pepstain A for 2 hr with 2 buffer changes.
11. Transfer the nuclear protein to a microcentrifuge tube, spin at 4^{o}C for 5 min to remove debris.
12. Measure protein concentration using the Bradford reagent. Divide into ~10 µg aliquots, snap freeze and store at -80^{o}C.

Reference: Nagao, Goekjian, Hong, and Key (1993) Plant Molecular Biology 21: 1147-1162.

EMSA (Gel Retardation) Protocols, Li-cor method

5X TRIS-GLYCINE BUFFER (1 L) pH 8.5

Tris-base	30.28 g
Glycine	142.7 g
EDTA	3.92 g

Let the buffer stir for at least 1 hour and then slowly bring the pH to 8.5 using HCl

10X BINDING BUFFER

Tris-HCl pH 7.5	10 mM
EDTA	1 mM
Glycerol	60%
DTT	10 mM add prior to performing the reaction

Filter sterilize all your stock buffers before making the 10X binding buffer

10X ORANGE GEL LOADING DYE

Sucrose	65% (w/v)
Tris-HCl, pH 7.5	10 mM
EDTA	10 mM
Orange G	0.3% (w/v)

Gel solution (100 mL)

5X Tris-Glycine	20 mL
40% Acrylamide solution	10 mL
2% Bis-acrylamide	2.5 mL
Glycerol	2.5 mL
ddH_2O	to 100 mL

Gel Preparation

1. Prepare the desired quantity of gel solution (usually 100 ml is required for two 12 well gel).
2. Before making the gel solution, clean the gel tray, tray cover and combs using 70% ethanol. It is important not to have any particle on the surface (it will likely show up during gel scanning).
3. Filter the gel solution through a 0.45 μm filter
4. Add 500 μL of freshly prepared 10% ammonium persulfate, mix thoroughly and then add 50 μl of TEMED. Mix thoroughly.
5. Pour the gel solution to the gel casting tray, and slowly put the cover on, making sure not to introduce any bubbles. Insert the comb, again, taking care not to introduce any bubbles. Your pouring surface should be level.
6. Let your gel polymerize for 1-2 hours. If you don't intend to run your gel immediately, cover it with plastic wrap to prevent it from drying out and use within a day (best within 12 hrs).

Preparation of Oligo for EMSA

7. IRDye labelled oligos can be ordered from LI-COR or other commercial vendors. Order both sense and antisense oligos

8. Oligo annealing: Add an equal volume and concentration of sense and antisense oligos into one tube. Oligos are annealed to form double stranded DNA by placing the oligo mixture on a 100°C heating block for five minutes. Leave the oligonucleotides on the heat block and turn off the heat to allow the mixture to cool slowly to room temperature.

9. Once cooled, spin down the oligos and mix before diluting to your working concentration. This step is determined by serial dilution of the oligos and running the various concentrations on a gel (as above) followed by scanning to determine the desired concentration for each oligo based on scanning intensity. It's important to make serial dilution (1:2), check by scan, and adjust if necessary. Do Not make dilution straight out of concentrated stock (like 1:10), as the concentration estimate is not always reliable.

DNA-Protein Binding Reaction

10. Perform binding reactions for 30 min at room temp as follows:

Oligo (double-stranded)	1 pmol/µL
Nuclear proteins	10 µg
10X Binding buffer	2.5 µL
Poly (dI-dC)	2 µg
PMSF	0.5 mM
ddH_2O	to 25 µL

For competition experiments, add unlabeled oligos at the desired molar ratios

Electrophoresis

11. Always run your gel at 4°C (in the cold room). Use cold 1X Tris-glycine buffer.

12. Before you remove the comb, add a little running buffer around the wells and then slowly remove the comb. This will help maintain the integrity of your wells. Pre-run the gel (50 V) for about 15 minutes before loading samples. Gels can be run between 50 and 75 V. Never run the gel at 100 V because this will generate a lot of heat which will dissociate the DNA-protein complex.

13. Scan the gel using the Li-Cor Odyssey according to the manufacturer's instructions.