Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Davison Life Sciences Building, Lab B310

Chemical(s)	Potassium ferrocyanide, Potassium ferricyanide, EDTA (ethylenediaminetetraacetic acid), Ascorbic Acid, PVP (Polyvinylpyrrolidone), X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid), DMF (Dimethylformamide), Acetone, Ethanol.
Process	**GUS Staining Protocol**
Specific Hazards *referred to MSDSs for more detailed information*	Ascorbic Acid: Slightly hazardous. Irritant. PVP: Irritant. Hygroscopic. Do not breathe dust. DMF: Irritant. Toxic. Possible reproductive toxin. Acetone, Ethanol: Flammable. X-Gluc: Irritant.
Personal protective equipment	☒ 3-5 mil nitrile gloves ☒ double gloves (w/ concentrated stock) ☒ lab coat (except when used in microcentrifuge tubes) ☒ chemical safety goggles (when splash potential exists)
Engineering/ventilation controls	Emergency shower and eyewash accessible. Chemical fume hood (when working with large quantities)
Special handling procedures and storage requirements	X-Gluc: stored @ -20°C, light sensitive. K-ferrocyanide, K-ferricyanide: stored @ 4°C, light sensitive. PVP: stored @ 4°C. DMF, Acetone, Ethanol: flammable cabinet, Rm B310, store away from oxidizers. Ascorbic Acid: Air and light sensitive.
Spill and accident procedures *for hazardous chemicals only*	Skin exposure: Rinse affected skin with plenty of water while removing contaminated clothing and shoes. Rinse for at least 15 minutes. Seek medical attention, as appropriate. Eye exposure: Wash eyes for at least 15 minutes, lifting the upper and lower eyelids. Seek medical attention, as appropriate.
	Small (< 2L): 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, notify PIs and call 2-5801 to request emergency assistance from Environmental Safety Division
Waste disposal	DMF, and Acetone wastes must 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.
Prepared by	Name/date: C-J Tsai, 8/8/09
Reviewed by	Name/date: Kate Tay, 9/2/09

GUS Staining Protocol

Reagents and Buffers:

1. 50 mM Potassium ferrocyanide (M.W. = 422.39): 0.2110 g in 10 mL of ddH_2O, Stored @ 4°C, Dark.
2. 50 mM Potassium ferricyanide (M.W. = 329.25): 0.1645 g in 10 mL of ddH_2O, Stored @ 4°C, Dark.
3. 1 M Potassium phosphate buffer (pH 7):
   3.9 mL of 1 M monobasic (KH_2PO_4) + 6.1 mL of dibasic (K_2HPO_4). **Don't use a pH meter.**
4. 0.5 M EDTA
5. 0.5M Ascorbic Acid – Sodium salt (M.W. = 198.1):
   0.9905g in 10ml of ddH_2O, aliquot in amber tube, Stored @ -20°C, Dark.
6. 10% PVP: 1g in 10 ml of ddH_2O, Stored @ 4°C, Dark.
   * Use ONLY the soluble PVP (Sigma PVP40T), NOT the poly-PVP.
7. 20 mM X-Gluc (M.W. = 521) in DMF:
   Add 1.92 mL of DMF directly to the 100 mg vial of X-gluc vial to make 100 mM stock.
   Dilute 5X with DMF to make 20 mM stock. Stored @ -20°C, Dark.
   X-Gluc is expensive; do not attempt to weight a sub-portion from the vial (wasteful).
8. Triton X-100
9. 90% Acetone (dilute before use)
10. 70%, 50%, 30%, EtOH.

X-Gluc Staining buffer: FRESH (1ml for 4x 96-well)

Component	Stock	Final	For 1 mL
ddH_2O	--	--	759 µL
K-phosphate, pH7	1 M	100 mM	100 µL
EDTA	0.5 M	10 mM	20 µl
Triton X-100	--	0.1%	1 µL (pipet slowly)
PVP #	10%	2%	200 µl
K-ferrocyanide#	50 mM	0.5 mM	10 µL
K-ferricyanide#	50 mM	0.5 mM	10 µL
Ascorbic Acid – Na salt##	0.5M	25mM	50 µl
X-Gluc##	20mM	1mM	50µl

Stored @ 4°C, Dark,
Stored @ -20°C, Dark.

Washing buffer: 100 mM K-phosphate, pH 7 (dilute 1 mL of 1M stock with 9 mL of ddH_2O)

Procedures:

1. Use a sharp razor to dissect tissues into pieces of less than 5 mm in any dimension.
2. Submerge immediately in 90% acetone (to fix protein) for exactly 30 min (use a timer to record the interval between each tissue dissection when processing multiple samples).
3. Rinse thoroughly with 100 mM potassium phosphate buffer (pH7), 3 times.
4. Add enough (e.g., 250µl) of **FRESH** X-Gluc staining buffer into an appropriate microplate (e.g., 96-well format) and make sure tissues are submerged in the staining solution (while conserving X-gluc).
5. Apply a mild/water vacuum for 10 min to facilitate infiltration.
6. Incubate @ 37°C for 12 hrs (monitor periodically, stop with sign of tissue browning)

7. Discard staining solution and incubate tissues in 70% EtOH to remove chlorophyll at room temp or 37°C. Change the EtOH solution once or twice until all chlorophyll is clear. Tissues can be stored in 70% EtOH (easy to dry out) or water (may get moldy).
8. Before doing vibratome sectioning, rehydrate the tissues through 50% EtOH → 30% EtOH → ddH_2O gradually, 1 hr each (to avoid damaging tissue structure).
9. Make cross section of 70-100 µm thick using a vibratome and seal the slide with nail polish
10. Observe under a microscope.