Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Davison Life Sciences B310

Chemical(s)	1-Butanol, Hydrochloric Acid, Chloroform, Ferric Ammonium Sulfate, Methanol
Process	**Condensed Tannin Assay**
Specific Hazards *referred to MSDSs for more detailed information*	Hydrochloric acid is corrosive and liquid or vapor may cause burns to skin/eye. 1-Butanol is flammable, and may be harmful upon skin/eye contact, ingestion or inhalation. Chloroform is a toxic solvent and contact to skin or eyes is dangerous. Butanol fumes are toxic. Methanol is flammable and harmful upon ingestion
Personal protective equipment	Must wear 3-5 mil nitrile gloves. Chemical safety goggles and lab coat should be worn when splash potential exist
Engineering/ventilation controls	All operations involving chloroform, hydrochloric acid and butanol must be done in a chemical fume hood.
Special handling procedures and storage requirements	Store chloroform, 1-butanol and methanol in the flammable cabinet, and hydrochloric acid in the designated cabinet underneath the chemical hood in Rm B310. Store all the other buffers on bench top.
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 2-5801 to request emergency spill assistance from Environmental Safety Division.
Waste disposal	Chloroform and 1-butanol waste must be collected and labeled as separate hazardous wastes according to the SOP for Hazardous Waste Disposal.
Special approval	No special authorization needed after SOP training & reading MSDSs.
Prepared by	Name/date: SA Harding, 8/18/2008.
Reviewed by	Name/date:

Condensed Tannin (CT) Assay Method

Solutions and Reagents needed

Butanol-HCl mix (5% HCl in 1-butanol): Slowly add 10 ml concentrated hydrochloric acid (HCl) to 190 ml 1-butanol in a glass-stoppered storage bottle in the fume hood. Swirl to mix.

FAS (2% ferric ammonium sulfate in 2M HCl): In the fume hood, add 2 ml HCl to 10.1 ml H_2O. Mix, and then add 210 mg ferric ammonium sulfate until completely dissolved.

BFAS (Butanol-FAS mix): Just prior to use, in the fume hood, add 1 ml of FAS to 50 ml of the Butanol-HCl mix. Mix well.

Chloroform, Methanol, heating block

Precautions: Gloves should be worn throughout the procedure. Goggles should be worn when working with chloroform, butanol or hydrochloric acid.

Procedure:

1. Leaf discs (fresh or freeze-dried) or tissue powder (freeze-dried) can be used. Use a standard paper hole puncher (1/4") to collect leaf discs into an Eppendorf tube containing methanol (100ml/disc). 2-4 discs, or 2-10 mg freeze-dried powder should be sufficient. Sonicate in a bath sonicator for 30 min. Transfer the de-pigmented discs to a new tube. Re-extract the tissue if green remains.

   If the tissue being measured does not have chlorophyll (e.g., roots or twigs), the following chlorophyll removal steps (#2) can be skipped.

2. Add 1.5 Vol chloroform and 2 Vol H_2O (**chloroform first, then H_2O**) to the methanol extract. Vortex and centrifuge at 10-15,000xg for 1 min to separate phases. Transfer the clear supernatant to a new tube, and dispose the lower chloroform phase as organic waste. Add small amounts of H_2O if separate phases do not form.

3. Evaporate the de-pigmented discs/powder (containing "insoluble" CTs) and the clear supernatant fraction (containing "soluble" CTs) to dryness using a Centrivap or equivalent.

4. CT color assay. To dried tissue and/or extract (two different assays), add 500ml of the BFAS solution. Close the microtube lid and attach a lid-lock. Heat the tube at 95^{o}C for 20 min. Cool to room temperature and read Abs_{550}. If quantitative rather than comparative results are desired, a standard curve using purified CTs will have to be prepared. If the color is dark, dilute accordingly using Butanol-HCl (NOT BFAS, water or methanol) and repeat the measurement.