



Safe Method of Use 23 – Disposal of DAB by Inactivation with Horse Radish Peroxidase and Hydrogen Peroxide

Purpose: This Safe Method of Use applies to **principal investigators (PIs), sector managers, designated laboratory person (DLPs)**, technical staff and students who use Diaminobenzidine within the University of Auckland.

This method of DAB disposal produces an insoluble product which can be filtered out of solution for disposal. The product is still mutagenic and must not be flushed down the sink.

Treatment of DAB with hypochlorite solution (bleach) also produces a mutagenic product in solution and is not a suitable method of deactivation.

DAB (3,3-diaminobenzidine tetrahydrochloride dehydrate) is toxic by ingestion, inhalation and skin contact. Long term exposure may cause cancer. Always use in a fume hood, wear gloves, lab coat and safety glasses. Neutralise solutions, wipe down surfaces and equipment in contact with DAB. Reduce contact with DAB by either using Sigma Fast tablets or making up bulk stock solutions of DAB and freezing. Read the MSDS before first use.

Hydrogen Peroxide (H_2O_2) 30% is toxic by inhalation, harmful if swallowed and causes burns to eyes and skin. It is an oxidising agent and is corrosive. There is limited evidence of a carcinogenic effect. Always use in a well ventilated area or fume hood, wear gloves, lab coat and safety glasses. Keep away from heat and store away from direct sunlight. Read the MSDS before first use.

Preparation of Reagents

Horse Radish Peroxidase - Sigma cat # P8250 50U – Dissolve in 83ml of dH₂O, aliquot into 0.5ml lots and freeze – this makes enough for 1L of DAB solution per aliquot.

3% H₂O₂ – add 5ml of 30% H₂O₂ to 45ml of dH₂O. Store in fridge for up to 1 month.

Deactivation of DAB

1. Add dH₂O to DAB waste and make to 1L in the suction flask
2. Add 1 aliquot (300 units) of HRP to solution
3. Add 3ml of 3% H₂O₂ to solution
4. Stand for 3 hours or overnight
5. Filter precipitate out using common grade filter paper
6. Dispose of precipitate in yellow Mediwaste bag