

Chemical Risk Management Protocol

Safe Methods of Use (SMOU)

Deactivation of Diaminobenzidine (DAB) with Horse Radish Peroxidase and Hydrogen Peroxide

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1 Purpose

This Safe Method of Use (SMOU) applies to principal investigators (PIs), laboratory managers, designated laboratory person (DLPs), and all staff and students who direct or participate in the use of diaminobenzidine at the University of Auckland.

Note: the word 'shall' denotes a mandatory requirement and the word 'should' denotes a recommendation.

2 Disclaimer

The Safety Data Sheet (SDS) should be consulted for specific information about the specific chemical formulation/concentration you will be using. The Gold FFX SDS Database is available on the Library database. Instructions on how to source this information can be found on the Health, Safety and Wellbeing Databases website:

<https://www.auckland.ac.nz/en/health-safety-wellbeing/health-safety-topics/laboratory-safety/chemical-safety/databases.html>

Please read this SMOU in conjunction with the Chemical Risk Management Guidelines.

Note: 'Shall' denotes a mandatory requirement and 'should' denotes a recommendation.

3 Important notes

This method of DAB disposal produces an insoluble product which can be filtered out of solution for disposal. The insoluble 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.

4 Safety considerations

DAB (3,3-diaminobenzidine tetrahydrochloride hydrate) 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 SDS before 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.

- **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 SDS before use.

5 Preparation of reagents

Horse Radish Peroxidase (HRP) - Sigma-Aldrich cat # P8250 50U – Dissolve in 83ml of distilled H_2O , aliquot into 0.5ml lots and freeze – this makes enough for 1L of DAB solution per aliquot.

3% H_2O_2 – add 5ml of 30% H_2O_2 to 45ml of distilled H_2O . Store in fridge for up to 1 month.

6 Deactivation of DAB

1. Add distilled H_2O to DAB waste to make it up to 1L
2. Add 1 aliquot (300 units) of HRP to the solution
3. Add 3ml of 3% H_2O_2 to the solution
4. Stand for 3 hours or overnight
5. Filter precipitate out using common grade filter paper
6. Collect precipitate and filter paper in a container for disposal by an approved chemical waste contractor. Wipe filter funnel with a paper towel to remove all traces of precipitated DAB and place with the rest of the solid waste.
7. Flush the solution obtained from precipitation down the sink with a large volume of water.