

Standard Operating Procedure (SOP) Template



DIAMINOBENZIDINE (DAB)

Effective Date: 1/18/2012

Revised Date: 1/31/2012

INTRODUCTION

A Standard Operating Procedure (SOP) describes how your lab will handle a hazardous chemical safely, including the amount and concentration you will use, how you obtain or create the working solution, and special handling procedures, engineering controls, and personal protective equipment. Chemical-specific SOPs are found on the [EH&S website](#).

Wake Forest Baptist Medical Center has created Standard Operating Procedures (SOPs) for several chemical hazard categories and some commonly-used chemicals. The SOP provides only standard information and requires customization for each lab.

The Occupational Safety and Health Administration (OSHA) requires a written SOP for any work with hazardous chemicals in laboratories. There are additional requirements for SOPs for particularly hazardous substances, or PHSs. These SOPs are an important part of the [Chemical Hygiene Plan](#).

GENERAL LAB RULES

1. No eating, drinking, smoking, handling contact lenses, or applying cosmetics in the laboratory.
2. Persons shall wear buttoned lab coat, long pants, safety glasses or goggles and appropriate gloves when working with hazardous chemicals.
3. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
4. All procedures are performed carefully to minimize the creation of splashes or aerosols.
5. Wash hands
 - after handling chemicals materials,
 - after removing gloves, and
 - before leaving the laboratory.
6. Plastic ware should be substituted for glassware whenever possible

Add Lab Specific Rules Here

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PURPOSE

- Use of 3, 3-Diaminobenzidine (DAB) as a brown stain in microscopy and immunohistochemistry.
- To reduce personnel exposure to Diaminobenzidine (DAB), it is recommended to use DAB Easytablets, Fisher catalog number: AC32800-5000; VWR catalog number: 200057-400 or Sigma catalog number: D3939.
- An alternative for DAB, which can be evaluated as a possible replacement, is Hanker Yates Reagent from PolySciences, Inc., catalog number: 08661. Hanker Yates Reagent produces a blue reaction product. Hanker Yates reagent is not mutagenic or a potential carcinogen.

PHYSICAL HAZARDS

- Incompatibilities and Reactivities: Metals

HEALTH HAZARDS

- Eye: Causes eye irritation.
- Skin: Causes skin irritation.
- Ingestion: May cause irritation of the digestive tract. The toxicological properties of this substance have not been fully investigated. May cause nausea and vomiting.
- Inhalation: Causes respiratory tract irritation. May cause irritation of the respiratory tract with burning pain in the nose and throat, coughing, wheezing, shortness of breath and pulmonary edema.
- Target organ: Blood.
- Mutagen.
- Suspect Human Carcinogen.

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PERSONAL PROTECTIVE EQUIPMENT

EYE PROTECTION

- Safety glasses, goggles or face shields shall be worn during operations in which DIAMINO BENZIDINE (DAB) might contact the eyes (e.g., through vapors or splashes of solution).
- Ordinary (street) prescription glasses do not provide adequate protection. Adequate safety glasses must meet the requirements of the Practice for Occupational Education Eye and Face Protection (ANSI Z87.1-1989) and must be equipped with side shields.
- Wearing contact lenses under some circumstances provides workers with a greater choice of eye and face protection (such as goggles or full-facepiece respirators without prescription inserts) as well as better visual acuity. However, the risk is unknown for contact lens wearers compared with nonwearers working with chemicals listed in the NIOSH Pocket Guide to Chemical Hazards [NIOSH 2004]. OSHA recommends against contact lens use when working with acrylonitrile, methylene chloride, 1,2 dibromo-3-chloropropane, ethylene oxide, and methylene dianiline." [NIOSH Publication No. 2005-139: Current Intelligence Bulletin 59, Contact Lens Use in a Chemical Environment](#)

HAND PROTECTION

- Use disposable nitrile gloves when working with chemicals.
- Laboratory personnel should thoroughly wash hands with soap and water before and immediately upon removal of gloves.

LAB COATS, ETC.

- Button lab coats, closed toed shoes, long pants and long sleeved clothing shall be worn when handling DIAMINO BENZIDINE (DAB). Protective clothing shall be worn to prevent any possibility of skin contact with DIAMINO BENZIDINE (DAB).

SAFETY SHOWER/EYEWASH

- Where the eyes or body of any person may be exposed to DAB, suitable facilities for quick drench or flushing of the eyes and body must be provided within the work area for immediate emergency use.
- Bottle type eyewash stations are not acceptable.

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DESIGNATED AREA FOR USE AND CONTAINMENT DEVICES

- All DIAMINOBENZIDINE (DAB) work shall be done in the laboratory fume hood. When working with volatile, toxic chemicals, in limited amounts, Biological Safety Cabinets (BSC) Class II, Type A2 exhausted or Class II, Types B1 and B2 BSCs exhausted to the outside can be used.
- The fume hood's sash must be in the position where a face velocity of 100 feet per minute is achieved. The BSC must be certified annually by a qualified outside vendor.
- Environmental Health and Safety certifies fume hoods annually and places a yellow sticker to indicate the date of certification and the proper sash height for achieving proper fume hood face velocity.
- Contact Engineering (716-4351) immediately if fume hood is malfunctioning.

SPECIAL HANDLING PROCEDURES AND STORAGE REQUIREMENTS

Handling: Avoid breathing dust, vapor, mist, or gas. Avoid contact with skin and eyes.

Add Lab Specific Special Handling/Storage Procedures

EMPLOYEE EXPOSURE MONITORING

- Users of DIAMINOBENZIDINE (DAB) are required to notify EH&S at 716-9375 so air sampling/monitoring may be performed to determine exposure level.
- Individuals planning a family or pregnant can contact EH&S for exposure determination, consultation, and recommendations.
- Exposure monitoring through EH&S is free of charge.

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CHEMICAL USE WITH ANIMALS

Click here to enter text. Please consider other routes of exposure when handling animal (e.g., cage sign and) and animal bedding

WASTE DISPOSAL

- Chemicals shall not be drain disposed.
- Excess DIAMINO BENZIDINE (DAB) and all waste material containing DIAMINO BENZIDINE (DAB) must be placed in an unbreakable secondary container labeled with the following “**HAZARDOUS WASTE DIAMINO BENZIDINE (DAB)**”.
- Full containers of DIAMINO BENZIDINE (DAB) waste must be disposed of according to the [EH&S Hazardous Waste Program](#).
- A [Waste Ticket](#) must be completed and submitted prior to disposing of DIAMINO BENZIDINE (DAB) through EH&S.

Decontamination of Equipment and Area

Glassware:

- Chlorine bleach is NOT effective in removing the mutagenic properties of DAB.
- Potassium Permanganate Method.

The most commonly used one currently involves potassium permanganate and sulfuric acid. End products are known to be non-mutagenic.

- Take up bulk quantities of diaminobenzidine tetrahydrochloride dehydrate in water and bulk quantities of the free base in 0.1 M hydrochloric acid so that the concentration of DAB does not exceed 0.9 mg/ml. Dilute solutions with the same buffer, if necessary, so that the concentration does not exceed 0.9 mg/ml.
- For each 10 ml of solution, add 5 ml of 0.2 M potassium permanganate solution and 5 ml of 2 M sulfuric acid solution.
- Allow the mixture to stand overnight, decolorize by the addition of sodium absorbate, neutralize and dispose solution down the drain with copious amounts of water.

Contact WFUSM EH&S at 716-9375 for further decontamination information.

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EMERGENCY PROCEDURES

Emergency Numbers:

| | |
|---|---|
| Fire and Medical Emergencies | 716-9111 (9 + 911 for Friedberg and PTRP campuses) |
| Principal Investigator's Emergency Number | Click here to enter text. |
| Employee Health | 716-4801 |
| Hospital Emergency Room | 716-9008 |
| Environmental Health and Safety | 716-9375 |

Laboratory Contact Information:

| | |
|---------------------------|---------------------------|
| Click here to enter text. | Click here to enter text. |
| Click here to enter text. | Click here to enter text. |
| Click here to enter text. | Click here to enter text. |

FIRST AID

- The nearest safety shower station is located: Insert text here
- The nearest safety eyewash is located: Insert text here

If chemical exposure occurs, flush exposed area for 15 to 20 minutes using emergency eyewash station and/or safety shower.

- Call 716-9111 and describe the extent of injuries, chemical spilled and the amount.
- Keep people out of the area.
- Report all accidental exposures to Employee Health Services.
- Complete an [online injury/illness report](#) if there is an over-exposure to the chemical or if there is an accident involving the chemical.

SPILL AND ACCIDENT PROCEDURES

If the chemical spilled is considered a carcinogen, reproductive toxin or highly toxic chemical, contact 716-9111 and evacuate area immediately, regardless of spill amount. If you are unsure of type of chemical, contact 716-9111. For all other spills use the chart below for spill reporting and response: [Chemical Spill Procedures](#)

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| SPILL QUANTITY | PROPER SPILL RESPONSE |
|---------------------------|---|
| Spill less than 300 mL | Contact Environmental Health and Safety (716-9375) and clean up spill using spill kit. |
| Spill greater than 300 mL | Do not attempt to clean up spill. Leave the Area and immediately report to WFBMC Security (716-9111). |

CERTIFICATION OF APPLICATION

PLEASE READ

THESE ITEMS AUTOMATICALLY BECOME A PART OF YOUR SOP

1. I understand that it is my responsibility to assure that all personnel working in my laboratory with any of these hazards are fully informed about their specific dangers, proper actions for safe use, steps to take in case of accidents, and are provided with all necessary safety equipment and instructions in its use.
2. I agree to follow the provisions of the [Chemical Hygiene Plan](#).
3. I will ensure that all of my personnel attend Basic Lab Safety Training by EH&S prior to using chemical materials.
4. I agree to permit Representatives of WFSM Environmental Health and Safety to inspect the facilities where this work is being conducted.
5. All chemical waste will be disposed of through EH&S Chemical Waste Program. I understand that **drain disposal is NOT allowed**.
6. Chemical materials will be transported in closed containers.
7. Sharps and/or breakable plastic solid waste items will be placed in approved puncture-resistant container, i.e., a sharps container.
8. As soon as possible, the Chemical Occupational Hygiene Officer will be notified in writing of any proposed changes in locations where chemical materials are stored or used.
9. Additional chemicals or changes in possession limits will be requested in writing from the Chemical Occupational Hygiene Officer.
10. Chemical materials will not be transferred to other Authorized Users without prior approval of the Chemical Occupational Hygiene Officer.
11. Chemical materials will not be shipped anywhere off campus without prior approval of the Chemical Occupational Hygiene Officer.
12. Secure chemicals (including waste) to prevent unauthorized access or removal. In addition, you must control and maintain constant surveillance of chemicals that are not in storage or are in use. This can be achieved by: 1) Locking refrigerators and/or storage cabinets, 2) Locking the laboratory when no one is present, and 3) Challenging unknown persons entering the laboratory.
13. I will notify the Chemical Occupational Hygiene Officer of my intent to leave WFUHS at least **60 days** in advance. I will be responsible for disposing of my chemical materials inventory through EH&S Chemical Waste Program.

| First | Last | Signature |
|---------------------------|---------------------------|-----------|
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