Title: Safe Use of Diphtheria Toxin

Physical Characteristics: Diphtheria toxin (DT) is the toxin produced by Corynebacterium diphtheria. DT is a 63 kDa dimeric protein composed of a 24 kDa A subunit (fragment) and a 39 kDa B subunit linked by disulfide bonds. DT binds to cell surface receptors leading to ADP-ribosylation of elongation factor-2 (eEF-2) and inhibition of translation.

Health Hazard Summary: DT is a potent and lethal toxin. A lethal human dose can be as little as 0.1 ug of DT per kg of body weight. Systemic DT can cause organ necrosis and death. Contact, inhalation, or oral exposure to DT can cause irritation and fever. Systemic exposure to DT, such as an injection into the bloodstream or an intramuscular injection, can cause fever or death.

Material Safety Data Sheet: Read attached MSDS.

Personnel Requirements:
All laboratory members that will be working with DT should have an up-to-date tetanus-diphtheria toxoid (Td) or its equivalent, such as, tetanus-diphtheria-acellular pertussis (Tdap) immunization. If it has been 2 or more years since the latest Td immunization, the person should be vaccinated with Tdap. Td immunizations will be made available if needed.

First Aid Procedures:
For oral exposure or if DT has been swallowed and if the person is conscious, wash out mouth with water and seek immediate medical care (telephone 2-4490). If person is unconscious, seek immediate medical care (telephone 2-4490).

For inhalation exposure, move person to fresh air and if breathing becomes difficult seek immediate medical care (telephone 2-4490).

For dermal exposure, rinse area with copious amounts of water for at least 15 minutes, remove any contaminated clothing, and seek medical care or advice (telephone 2-4490).

For contact exposure to the eye, flush eye with copious amounts of water for at least 15 minutes and seek medical care or advice (telephone 2-4490).

For systemic exposure, seek immediate medical care (telephone 2-4490).

Any exposure incident requires the completion and submission of a an incident
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report through https://MyREHS.rutgers.edu to document the event..

Exposure Control:
Avoid inhalation or physical contact with DT.
Use only in a chemical fume hood.
Use physical barriers to exposure such as gloved hands, safety glasses, and lab coat.
Insure that safety shower and eyewash station are nearby.
Purchase in small quantities.
Aliquot any stock solutions into smaller 1 to 3 use aliquots.

In case of accidental release:
For small quantities (less than 5ml or 100 ug), while wearing gloves, lab coat, and safety glasses/goggles.

- Liquids should be absorbed with spill pillow; solids should be wiped up with wet absorbent gauze. The spill areas should then be cleaned three times using a detergent solution and paper towels followed by clean water.
- Any broken glass fragments should be picked up using a small scoop (never the hands) and placed in a wide-mouthed plastic container. The container should then go into a hazardous drug disposal bag, along with used absorbent pads and any other contaminated waste.
- A trained employee wearing two pairs of nitrile gloves, eye protection and fully fastened lab coat or gown should wash contaminated reusable items, (e.g., glassware and scoops) three times with detergent.
- Spill clean-up waste (paper towels, absorbent pads, gloves, spill pillows) must be bagged and autoclaved at 121 degrees C and 15 psi for 60 minutes on liquid cycle (slow exhaust). The materials will then be disposed of as medical waste.
- Report a small spill by notifying Public Safety (2-4490) that a small spill has occurred, and trained personnel are cleaning it up.
  - Be prepared to provide the following information:
    ▪ Phone Number where knowledgeable person can be contacted.
    ▪ Name of chemical
    ▪ Number injured, if any
    ▪ Amount spilled
    ▪ Location of Spill
- Public Safety will contact the on-call REHS staff member, and provide them with the reported information.

For larger spills (greater than 5ml or 100 ug) or if there is a possibility that an airborne powder or aerosol has been generated, REHS should be notified, as the clean-
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up will require the use of a NIOSH-approved respirator. Evacuate the area, and wait for REHS to respond.

- Report a large spill by notifying Public Safety (2-4490)
  - Be prepared to provide the following information:
    - Phone Number where knowledgeable person can be contacted
    - Name of chemical
    - Number injured, if any
    - Amount spilled
    - Location of Spill
- Public Safety will contact the on-call REHS staff member who will arrange for the proper clean up and disposal of the spilled materials.

Materials:
1. Diphtheria Toxin from Enzo Life Sciences, catalog number BML-G135-0500, quantity 500 ug.
2. DT comes as a lyophilized powder.

Preparation:
1. In a fume hood while wearing gloves, lab coat, and safety glasses, inject 500 ul of water into the DT vial and mix.
2. Open vial with forceps, not your fingers.
3. Prepare 25 ul aliquots in labeled 15 ml conical centrifuge tubes or labeled 1.5 microcentrifuge tubes; each aliquot contains 25 ug of DT.
4. Store aliquots at -80°C.

Procedure for Use in Mice:
1. Be extremely cautious using needles with DT.
2. In a fume hood while wearing gloves, lab coat, and safety glasses, add 10 mls of saline to a 25 ug aliquot of DT and mix to bring to 2.5 ng/ul.
3. Draw an aliquot of the diluted DT into a 1 cc tuberculin syringe.
4. In the fume hood, intraperitoneally inject 10 ul of diluted DT (2.5 ng/ul) per gram of body weight into the mouse (a 20 g mouse would be injected with 0.2 cc of diluted DT).
5. Mark cage to indicate that mice in the cage have been treated with DT.
6. Store unused DT at -20°C and avoid repeated freeze-thaw cycles.
7. Repeat DT dosing every other day for a maximum of 5 doses as above.
8. Sacrifice mice as per normal for specimen collection, but continue to use gloves, safety glasses, and lab coat.
9. Place euthanized, DT treated mice in a separate bag and label. Return to research animal facility for disposal.
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Procedure for Use in vitro:
1. In a fume hood while wearing gloves, lab coat, and safety glasses, add 975 uls of sterile saline to a 25 ug aliquot of DT and mix to bring to 25 ng/ul.
2. Store diluted DT at -20°C. Avoid repeated freeze-thaw cycles.
3. Use as a 1,000X stock solution, nominal ED50 for DT in cell culture is 0.4 ng/ml.
4. Collect culture media containing DT and inactive with bleach prior to disposal.

Signatures:
By signing below, I certify that I have read this SOP and attached material, that I understand the procedures for working with diphtheria toxin, that I understand the hazards associated with using diphtheria toxin, and that I will use the procedures described in this SOP to safely handle and use diphtheria toxin.

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Principal Investigator (print): _____________________________________
Principal Investigator Signature: ________________________________
Date: __________________________________________________________________

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