**Standard Operating Procedure**

# for native cholera toxin (composed of the A and the B subunits)

# from Vibrio Cholerae bacterium (CAS 9012-63-9)

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| **PI Name:** | **Building(s):** |
| **PI Signature:** | **Room Number(s):** |
| **Date:** | **Designated Work Area:** |
| **OESO Reviewer Name and Signature:** | **OESO SOP Approval Date:** |

1. **Hazard Identification**
   1. **Background and Risk Assessment**

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| This product is the active, native cholera toxin (composed of the A and the B subunits) from Vibrio Cholerae bacterium. Cholera toxin is the virulent factor from Vibrio cholerae that leads to severe diarrhea followed by dehydration in humans. Cholera toxin exposure can occur through the skin and eyes, and by inhalation or ingestion; exposure can cause irritation, vomiting, diarrhea, abdominal pain, and bowel failure, and may be fatal. Inhalation is possible when preparing a solution from the lyophilized powder, while skin exposure may occur during splashes while aliquoting solutions. Reports of toxicological data in mice show LD50 values 260ug/kg for i.v. exposure, and an LDlo value of 10ug/kg in monkeys via i.v exposure (RTECS No. LF3100000).  **HAZARDS IDENTIFICATION:**    Danger  Acute toxicity, Oral (**Category 2**), H300  Acute toxicity, Inhalation (Category 4), H332  Acute toxicity, Dermal (**Category 2**), H310  Specific target organ toxicity - repeated exposure, Inhalation (**Category 2**), Respiratory  Tract, H373  Short-term (acute) aquatic hazard (Category 3), H402  Long-term (chronic) aquatic hazard (Category 3), H412 |

* 1. **Preparation and Use:**

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| **Purpose for use:**  **Primary Dilutions\* for storage:**  **\*NOTE: Primary dilutions must be performed under a chemical fume hood or Class II Biological Safety Cabinet (BSC).**  **Note: In our lab \_\_\_\_\_ and/or\_\_\_\_\_are the only individuals to perform this task.**  Upon initial unpacking, this toxin will be resuspended to a \_\_\_\_) solution by injecting sterile water into the vial through the rubber stopper lid. The syringe with needle will be immediately disposed of in the sharps container that is contained within the hood or BSC. This solution will be pipetted and stored in aliquots of (\_\_\_\_) and stored (\_\_\_\_\_\_). Importantly, for aliquotting purpose, the rubber lid will be carefully opened and a pipet will be used to dilute, distribute, and aliquot this toxin solution into Eppendorf tubes.  **Subsequent OR Additional Dilutions for use in assay and/or Animal Work:**  **AFTER animals have been dosed, follow standard DLAR animal handling practices for ABSL1. Door signs and cage cards are not required for ABSL1 animal housing areas.** |

* 1. **Routes of Exposure and Potential Hazards:**

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| The primary risk of working with this toxin in the lab is **skin pricking with a needle or other sharps** which would allow entry of the toxin into the blood. **Exposure may also occur via inhalation**.  All routes of exposure include skin, inhalation, ingestion, and injection.  The risks **are greatest when working with the toxin in powdered form, working high concentrations (amount of toxin) and when performing injections.** |

1. **Hazard Control**
   1. **Selection and Purchasing:**

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| ***Vendor/Type (powder, liquid)/amount/concentration per container.*** |

* 1. **Engineering Controls:**

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| Within a Class II BSC, to prevent inhalation, the toxin will NOT be weighed, but will be dissolved directly in the vial as described above and stored at 4oC in its original container. The subsequent dilution (e.g. with saline before injection into animals) will also be performed in a Class II Biological Safety Cabinet (BSC) or the Chemical Fume Hood (CFH).  All work with preparation of toxins (from lyophilized powder) will be conducted within the operationally effective zone of the BSC or the CFH. Each user should verify the inward airflow before initiating work. Because this toxin is not an infectious agent, no specific biocontainment level is appropriate. Instead, specific precautions will be implemented that are specific to working with the toxin. Typically, these precautions are equivalent to Biosafety Level 2. |

* 1. **Administrative and Work Practice Controls:**

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| **Recombinant DNA work that encodes for toxins must be approved by the Institutional Biosafety Committee (IBC). See** [**IBC webpage**](https://www.safety.duke.edu/biological-safety/institutional-biosafety-committee-ibc) **for more information.**  The toxin in powdered form will never be handled in an open container.  The toxin in solution will be handled using disposable plasticware, which will be treated as biohazard waste after use. Specifically, no glass or pasteur pipettes or syringes will be used after the primary dilution.  Decontaminate the work surface of the BSC or CFH using 1:10 household bleach (0.5% sodium hypochlorite) after use. Rince surfaces to reduce corrosion.  To avoid accidental autoinoculation, **extreme care** will be exercised when handling the toxin in conjunction with any injection device.   * A hand-washing sink must be readily available to all locations where toxins are used. * An emergency shower and eyewash station or eyewash/drench hose combination unit is recommended in locations where toxins are used |

* 1. **Personal Protective Equipment (PPE):**

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| When working with the toxin in the laboratory, personnel will wear (at minimum) a lab coat, gloves, safety glasses, and a mask. If applicable: Within the animal facility, the standard gloves, mask, gown/coveralls, bonnet, and shoe covers will be worn, along with safety glasses. |

* 1. **Storage and Transportation:**

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| * Toxin must be placed in a secondary, shatterproof container and must be   TOXIN  labeled with hazard warnings at a minimum.   * When toxins are stored in the lab, containers must be sealed, legibly labeled and secured to ensure restricted access. * Use spill trays/plastic backed absorbent material (chux diapers) in fume hoods, BSCs, glove boxes, especially in solution. * Refrigerators and other storage containers containing biological toxins should be labeled with staff contact information (do not list the toxin name) for trained, responsible laboratory staff. |

1. **Emergencies, Spill Procedures, and Exposures/Unintended Contact**

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| *First Aid/Exposure Response*:   1. Skin Exposure/Wound: Immediately go to the sink and thoroughly wash the skin with soap and water. 2. Splash to Eye(s), Nose or Mouth (mucous membrane): Immediately flush the area with running water for at least 15 minutes. 3. Splash Affecting Garments: Remove garments that may have become soiled or contaminated and dispose as chemical waste. 4. In the event of an acute injury or overt exposure, the injured employee/student should **immediately inform their supervisor/PI and notify Duke Employee Occupational Health and Wellness (EOHW) via the Employee Exposure Hotline at (919)-684-8115 (115 campus landline).** EOHW may instruct the employee/student to report to the Duke Emergency Department (ED) for medical assessment and to take a copy of their laboratory protocol document to the ED including information about the dose associated with the exposure. 5. Prior to going to the Duke ED, a co-worker will be asked to secure toxin in locked storage, unless spilled, and secure the lab. If personnel are working alone, lab will be secured prior to going to the Duke ED. If the toxin is spilled, lab will be secured and the “*Spill Response Procedures*” below will be followed. 6. Follow-up is needed in the event of **any** exposure. The employee/student is to follow up at Duke EOHW after first aid/ED treatment on the same or next business day.   *BSC/Fume Hood Failure:*  Close or cover all toxin containers. Shut down operations, close hood sash, and evacuate room. Contact your maintenance provider to repair the BSC or fume hood.  a. Facilities Management Department: 919-684-2122 (University Buildings only)  b. Engineering and Operations: 919-684-3232 (Medical Center Buildings only)  *Spill Response Procedures:*  For spills of diluted toxin solution (inside or outside BSC):   1. If personnel are exposed/injured, follow “*First Aid/Expsure Response*”. 2. Inform others of the spill and mark area as SPILL, DO NOT ENTER. 3. At minimum, safety glasses, lab coat, smock, or coveralls should be worn, along with   appropriate gloves to clean up a spill. If splashing may occur, safety goggles and a face shield must be worn in place of safety glasses.   1. Use tongs/forceps to pick up glass, if applicable and place glass in a biohazard sharps container. Do not use hands to touch broken glass. 2. Cover spill with paper towel or other disposable, absorbent material. Material will be carefully wiped up, placed into an autoclavable biohazard bag. 3. Apply freshly prepared 1:10 bleach solution to the entire area, beginning at the perimeter and working towards the center, and allowing sufficient time (**30 minutes**) to completely inactivate the toxin. Wipe area with paper towel or other disposable, absorbent material and place into the autoclave bag. 4. Autoclave at 121°C and 15 psi for 90 minutes before disposal.   For spills of toxin powder:   1. If personnel are exposed/injured, follow “*First Aid/Expsure Response*”. 2. Inform others of the spill and mark area as SPILL, DO NOT ENTER. 3. Cover the spill with a freshly prepared 1:10 bleach moistened (wet) paper towel or other disposable, absorbent material. 4. Apply 1:10 bleach solution to the entire area, beginning at the perimeter of the spill and working towards the center, and allowing sufficient time (**30 minutes**) to completely inactivate the toxin. Place towels into an autoclavable biohazard bag. 5. Repeat Step 4 to ensure inactivation of toxin, as needed. 6. Autoclave at 121°C and 15 psi for 90 minutes before disposal.   **If at any time you do not feel qualified to clean up the spill according to the instructions above, contact the OESO Spill Response team by calling 911 from a campus phone or 919-684-2444 from any phone to report the spill.** |

1. **Waste**

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| **Liquids**: *may be chemically decontaminated* (at least one method must be listed):   * Name and concentration of decontamination solution: Use **1:10 bleach solution**, made daily * Time for effective decontamination: 30 minutes contact time with 1:10 bleach solution at room temperature will inactivate the toxin. After 30 minutes and while wearing face protection, pour down the drain followed by a copious amount of water.   All solid material to come into contact with this toxin will be disposed in biohazard bags and **autoclaved for 90 minutes at 121°C before disposal**. Used needles and syringes will be placed in red plastic sharps disposal containers, closed when 2/3 filled, and autoclaved for 90 minutes at 121°C. Animals treated with this toxin will be disposed by DLAR-standard disposal method. |

1. **Training**

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| All personnel who work with this toxin will be required to:   * Be trained in the theory and practice of the toxin to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations and accidental spills. * **Read and fully adhere to this SOP when handling this toxin.** * **Be current in laboratory worker health review from EOHW by making an appointment to discuss health considerations and risk (919-684-3136).** * Sign this SOP documenting compliance with these requirements. |

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from Vibrio Cholerae bacterium

**Signature Page:**

**“I have read and understand this SOP. I agree to fully adhere to its requirements.”**

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| **Last** | **First** | **Duke ID** | **Signature** | **Date** |
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