

Human Immunology Center Core Laboratory

David H. Smith Center for Vaccine Biology and Immunology

Aab Institute of Biomedical Sciences

STANDARD OPERATING PROCEDURE: Proper Use and Handling of Staphylococcal Enterotoxin B (SEB).

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Approval:

1. General Information

Staphylococcal Enterotoxin (SEB) is a single polypeptide chain containing 239 amino acids and is a member of the bacterial superantigen family¹. It is a biohazardous material and BSL 2 guidelines should be followed when working with the reagent. It may cause fever, or respiratory symptoms like coughing, dyspnea, retrosternal discomfort, chest pains or gastrointestinal symptoms if inhaled. If ingested, it may cause symptoms similar to those of food poisoning.¹ SEB is received as a lyophilized powder and diluted to a 1mg/mL solution in distilled water.

Supplier Information: **Sigma-Aldrich**
 3050 Spruce Street
 St. Louis, MO 63103, USA,
 1-800-325-5052
 Catalogue # S4881-1MG
 CAS#11100-45-1

2. Purpose for using this agent in the laboratory

SEB is used as a mitogen in immunological assays such as intracellular staining, cytokine secretion assay, ELISPOTS, and Fluorispots performed in the Center for Vaccine Biology and Immunology.

3. Ordering Information, Chain of Custody, and Storage

SEB will be ordered from the current preferred vendor Sigma Aldrich. HIC laboratory personnel will order the SEB when less than 50 mg of the reagent is remaining in the current working inventory. The maximum amount on hand will be 1.050mg. When placing an order, check current inventory to confirm that the ordered amount will NOT cause the laboratory/Principal Investigator to exceed the Exempt Quantity Threshold of 5mg for cumulative possession of the toxin. The maximum amount that can be ordered in the HIC lab will be 1mg. When placing the order, obtain an order confirmation number and anticipated shipping and arrival date from the vendor.

The SEB will be shipped from a Sigma facility following the appropriate laws as outlined by the DOT and AFTA. Only trained personnel within the HIC laboratory will receive the SEB. All personnel authorized to receive and/or handle SEB in the HIC laboratory must read the Material Safety Data Sheet for SEB found in the HIC laboratory safety binder.

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When the SEB is received in the HIC laboratory:

- a) Review the outer pack/shipping paper to confirm that the correct type and amount of SEB was received. If an incorrect order or amount has been received, contact the vendor. Also contact EH&S at ext. 5-3241.
- b) Post signage "Toxins in Use-Authorized Personnel Only."
- c) Inspect the outer package to make sure it is intact. If the outer package is intact, place the package in a biosafety cabinet before opening it. Verify that the airflow is functioning properly. In the BSC place a tray containing lab absorbent paper or paper towels to place the inner package on. If the outer package is NOT intact, place the package in a biosafety cabinet, and contact Security at ext. 13. Security will dispatch EH&S. Place a sign on the BSC to alert others to not use that equipment until further notice ["Toxins in Use-Authorized Personnel Only."]
- d) Put on the following PPE before opening the outer and/or inner packaging: lab coat, a double layer of Latex or Nitrile gloves (so that the outer layer of gloves can be removed and your hands still remain protected by one layer of gloves), safety glasses, and a mask. [Note: it is strongly recommended that you wear Tyvek sleeves when opening and handling a shipment, as the sleeves are disposable.]
- e) Open the outer packaging carefully, and inspect the toxin container. If the container is intact, follow step 7.b) of this Standard Operating Procedure.
Note: If the inner package is NOT intact, leave the package in the biosafety cabinet, and contact Security at ext. 13. Security will dispatch EH&S. Place a sign on the hood/BSC to alert others to not use that equipment until further notice.
- f) If the vial is not damaged, excess packing materials and the outer shipper box may be disposed of as regular trash. If the vials are damaged, see the Spill and/or Waste Disposal sections.
- g) Record the shipment in the SEB inventory log located in the Select Agent Binder [inventory log form provided by EH&S].

4. Inventory

The Principal Investigator is responsible for inventory control of the SEB. The PI may designate someone within the lab to perform this task. When a new vial of SEB is received in the laboratory, it will immediately be recorded on the SEB inventory log. As amounts of SEB are removed from the inventory, the volume being removed will be immediately recorded on the SEB inventory form (Subtract volume removed from total available). SEB must be counted cumulatively on the inventory log. The total amount of SEB on hand includes all forms of SEB including the original powder stock from the vendor, and all cumulative amounts already diluted or aliquoted for future experimental use. Inventories must be rectified quarterly (April, July, October and January). A visual verification of the inventory will be performed and verified against the inventory log.

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The verification will be recorded on the inventory log either by the PI or a person designated by the PI. The inventory sheet will be signed by the Principal Investigator and then faxed to EH&S by the 15th of each inventory month. The PI or a person designated by the PI must investigate all discrepancies in the inventory.

5. Security and Restricted Access

The laboratory doors in the HIC laboratory will be lock when HIC personnel are not in the laboratory for extended periods of time. The SEB will be stored within the laboratory in a locked -20°C freezer. Only HIC personnel will have access to the key for the freezer. A lab contact form will be posted outside of the laboratory with PI contact information. When SEB is received into the laboratory and reconstitution of the reagent is being performed, a sign will be placed outside the laboratory door indicating restricted access. All laboratory personnel will be notified that SEB is being reconstituted and there will be limited access in the area of the BSC. If a theft/loss of SEB is discovered, immediately contact EH&S at extension 5-3241 and security at X13.

6. Transfers

If a SEB is transferred or shared with individuals outside of the laboratory, the toxin will be placed in a plastic sealed bag labeled with a biohazard label and then placed inside a secondary leak proof container. The amount shared with other laboratory will only be the amount needed for one designated experiment and must be used immediately for that experiment. No storage outside of the HIC designated locked freezer is allowed. This amount will be recorded in the inventory log immediately. Excess amounts of SEB will not be released.

7. Use and Administration of the Toxin

a) Reconstituting or Diluting Original Stock Lyophilized Powder

- 1) Place a tray with bench coat/paper towels on it in the BSC. Confirm that the BSC is working properly
- 2) Post signage "Toxins in Use-Authorized Personnel Only."
- 3) Put on the following PPE: lab coat, a double layer of Latex or Nitrile gloves (so that the outer layer of gloves can be removed and your hands still remain protected by one layer of gloves), safety glasses, and a mask. [Note: it is strongly recommended that you wear Tyvek sleeves when opening and handling a shipment, as the sleeves are disposable.]
- 4) Set up necessary waste receptacles inside the BSC containing a 10% bleach solution.

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- 5) Remove the metal seal around the rubber stopper with a decrimper. Remove the rubber top and add 1mL of distilled water to the vial. Place the rubber cap on the vial and invert to mix. When aliquoting samples, avoid foaming or aerosolization. Prepare one hundred 10 μ L size aliquots.
 - 6) Label the aliquoted samples with the SEB, concentration, manufacturer, lot number, amount and date aliquoted.
 - 7) Place the aliquoted toxin vials in an unbreakable/break-resistant secondary container. Label the outside of the secondary container with the following information:
“Highly Toxic” and Staphylococcal Enterotoxin B/SEB, quantity/concentration.
 - 8) See “Waste Disposal and Toxin Inactivation” Sections for disposal procedures for the original container or any wastes generated.
- b) Diluted Stocks/Experimental Use Protocol
- 1) Place a tray with bench coat/paper towels on it in the BSC. Confirm that the fume hood or BSC is working properly.
Note: If the toxin must be worked with outside of hood or BSC containment fields, please contact EH&S (x5-3241) for a task-specific consultation.
 - 2) Post signage “Toxins in Use-Authorized Personnel Only.”
 - 3) Put on the following PPE: lab coat, safety glasses, and two layers of Latex or Nitrile gloves. Tyvek sleeves and a mask are recommended.
 - 4) Set up necessary waste receptacles inside the BSC containing a 10% bleach solution.
 - 5) Do not vortex SEB to limit aerosols. SEB will be contained within a BSC except during incubation times where it will be placed in an incubator (It will be contained within a 96 well plate with a lid. Remove the specified amount of SEB and dilute it as needed. Write on vial the amount of SEB removed. Place unused portion in labeled box in -20°C freezer. Each vial should only be thawed 4X. After the 4th thaw, discard remaining SEB as indicated in the waste disposal section of the protocol.
 - 6) Label the incubator with “Highly Toxic” and toxin name/abbreviation, quantity/concentration.
 - 7) After completion, spray the tray with 10% bleach solution and let sit for 30 minutes. Spray the BSC with vesphen and allow contact on the surface of BSC for at least 20 minutes.
8. Waste Disposal and Decontamination Procedures

All items (tips, pipets, assay plates) coming in contact with SEB will be decontaminated by placing the items in a container with 10% bleach for a minimum of 30 minutes. After

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30 minutes place items in a double biohazard bag and placed in the appropriate bin for disposal. All surfaces will be decontaminated with 10% bleach.

9. Spill Clean Up

Spills Outside of a Fume Hood or Biosafety Cabinet

- a) If a spill occurs outside of a fume hood or BSC, notify lab personnel and evacuate the immediate area until the extent of the spill is determined. Close the doors to the laboratory to prevent unauthorized access. Only authorized and designated persons may participate in spill cleanup.
- b) If it is a minor fluid spill, don appropriate personal protective clothing [double gloves, lab coat, mask, and safety glasses if splash hazards could occur], and cover the spill with absorbent material (e.g., paper towels).
- c) Soak the area with a 10% bleach solution and inactivate the toxin by allowing for at least a 30-minute contact time, remove absorbent material and dispose of in appropriate waste container [i.e., non-sharps in double biohazard bag, sharps in sharps containers]. Clean affected area 10% bleach. Also, wipe down any equipment, shelves, cupboards with a 10% bleach solution, and place contaminated towels and other non-sharp waste in a double biohazard bag.
- d) Major fluid spills, or release of the original lyophilized, granular or powder stocks must be called into Security (ext 13) for an EH&S response. No one must remain in the lab, and the lab must be posted for the spill until it is cleaned up.

Inside of a Fume Hood or Biosafety Cabinet

- a) If a spill occurs inside of a fume hood or BSC, notify lab personnel and determine the extent of the spill. Ensure that the doors are closed to prevent unauthorized access. The hood or BSC must be posted with proper signage until it is cleaned up. The lab does not have to be evacuated unless the hood/BSC cannot contain the spill.
- b) For minor fluid spills, don appropriate personal protective clothing, and cover the spill with absorbent material (e.g., paper towels). Then cover the spill/absorbent material with a 10% bleach solution. Allow for at least 30-minute contact time, remove absorbent material and dispose of in appropriate waste container [i.e., non-sharps in double biohazard bag, sharps in sharps containers]. Clean affected area with a 10% bleach solution. Also, wipe down any equipment, shelves, cupboards with the 10% bleach solution, and place contaminated towels and other non-sharp waste in a double biohazard bag.

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- c) Major fluid spills, or release of the original lyophilized, granular or powder stocks must be called into Security (ext 13) for an EH&S response.

10. Post-Exposure Actions

- a) After an accidental exposure contact through a cut, puncture, dermal/skin, mucous membrane, flush with copious amounts of water. Remove contaminated clothing, gloves, lab coats, etc., before leaving the immediate area. Call UHS (normal work working hours) or the Emergency Department (after hours).
- b) Contact Security at X13 for an EH&S response.
- c) If the material has become airborne, the employees and occupants must leave the immediate area, closing doors where possible. Signs must be posted indicating that there is a potential that the toxin has been aerosolized and not to enter the room. Contact Security at X13
- d) If there is potential skin, eye, oral, or face exposure, flush exposed or affected body with water from an eyewash and/or safety shower for at least 15 minutes.
- e) If potentially inhaled, injected, or absorbed through the skin, report immediately to UHS (normal work hours), or the Emergency Department (off hours).
- f) The affected employee's Supervisor must be immediately notified.
- g) The affected employee's Supervisor must complete and submit an incident report within 24 hours of the incident.

11. Reference

¹Rusnak JM, Kortepeter M, Ulrich R, Poli M, Boudreau E. Laboratory exposures to staphylococcal enterotoxin B. Emerg Infect Dis [serial on the Internet]. 2004 Sept [cited 03/05/06]. Available from: <http://www.cdc.gov/ncidod/EID/vol10no9/04-0250.htm>

CDC website www.cdc.gov