



Title: Preparation, Administration, Handling and Care of Streptozotocin (STZ)

SOP Number: 073

Purpose: This standard operating procedure is for use of Streptozotocin in animal research to induce diabetes.

BACKGROUND

Streptozotocin (STZ) is a probable human carcinogen and experimental teratogen. It accumulates in the liver, kidney and pancreas. After IV or intraperitoneal administration, 10 to 20% of STZ is secreted unmetabolized and 60 to 70 % is metabolized within 4 hours. It may be secreted for up to 24 hours. After 72 hours, administered STZ has dissipated from the animal. STZ decomposes to form diazomethane when exposed to alkaline pH. Diazomethane is acutely toxic and is an inhalation hazard. The use of N95 respirators is required during certain activities. Use of N95 respirators requires fit testing and training. Contact EHS to get respiratory fit testing set up prior to use.

STREPTOZOTOCIN (STZ) PREPARATION AND ADMINISTRATION MATERIALS

Streptozotocin

Glacial Acetic Acid

Anhydrous Sodium Acetate (or Sodium Acetate Trihydrate)

Sodium Chloride

Double distilled water (DDW)

Peroxigard or other appropriate disinfectant

Syringe filter (0.22 μ m)

Acetate Buffer (1mL per 5 mice or 1 rat)

Small red top vacutainers (one /rat or 5/mice)

1 mL syringes (one /rat or 5/mice plus one)

27 G needles (one /rat or 5/mice)

18 G needle (one)

ACETATE BUFFER PROTOCOL

1. Solution A: Measure 0.7775 mL glacial acetic acid and bring up to 50 mL with DDW.
2. Solution B: Weigh 0.82 g anhydrous sodium acetate OR 1.36 g sodium acetate trihydrate and bring up to 50 mL with DDW.
3. In a 50 mL volumetric flask, add 15.25 mL Solution A and 9.75 mL Solution B together. Bring to volume 50 mL with DDW and add 0.45 g NaCl to the solution.
4. pH the solution to 4.5 and sterile filter.
5. Store all solutions in dark bottle (or cover with tinfoil) and store at 4°C.

STREPTOZOTOCIN PREPARATION

1. Fill a bucket with ice and keep buffer cold until needed.
2. Allow streptozotocin to reach room temperature in a desiccator.

3. Inside of a biological safety cabinet or chemical fume hood aliquot the streptozotocin into the red vacutainers according to weight needed (e.g. 55 mg). *Adjust weight of STZ for different injection volumes.*
4. Place tubes that are to be used immediately on ice. All other tubes and remaining streptozotocin should be stored in a desiccator at -20°C

ADMINISTRATION

1. Animal may or may not be anesthetized using Isoflurane depending on competency of animal handler
2. Weigh animal and record weight.
3. Use the appropriate dosage.
4. Calculate amount of drug needed.
5. Take 1 mL of buffer and inject into vacutainer using a syringe with 18 G needle. Vortex for 30 seconds. (You may reuse this needle and syringe to add buffer for other tubes).
6. Withdraw STZ/buffer solution using a new syringe with 27 G needle and inject intraperitoneal/intravenous. **Watch the animal's breathing!**
7. Remove needle and hold pressure on injection site until bleeding stops.
8. Allow animal to awaken (if anesthetized) and dispose off all drug waste into labeled double biohazard bags. Needles go into regular sharps container.
9. Wipe Down any contaminated surfaces with Peroxigard.

ANIMAL EXPERIMENTS

- a) After administration of STZ, rodents will be placed in clean IVC cages with fresh alpha-dri bedding to prevent aerosolization, a clean wire bare lid, and clean water bottles that are full. IVC cages should not be opened during the first 48 hours. The first bedding change should be disposed of as only incinerated waste through EHS. In this scenario, routine change outs are superseded. If it becomes necessary to open IVC cages within the first 48 hours, they should only be opened inside a Biosafety Cabinet (BSC) or designated fume hood. If manipulations occur outside of a BSC or chemical fume hood during the first 48 hours, personnel must wear an N95 respirator plus Personnel Protective Equipment (PPE) listed in (d), below.
- b) Rodents must be injected with STZ within a BSC or designated fume hood.
- c) The room door must be clearly marked with a chemical hazard sign during the procedure.
- d) Animal handlers should wear PPE, including mask, lab coat or smock with long sleeves, double gloves pulled over the end of the coat or smock sleeves and shoe covers. A separate face shield is not required for personnel working in the hood who are protected by the hood shield.
- e) A biohazard bag must be placed inside the BSC for waste materials. A small sharps container should also be placed in the BSC. The sharps container will be placed in the

biohazard bag for incineration at the end of the procedure. All materials exposed or potentially exposed to STZ will be placed into the biohazard bag for incineration at the end of the procedure and the biohazard bag will be immediately processed for incineration.

- f) Clean areas where STZ has been handled using water and placing paper towels on top. Repeat at least 3 times and dispose off waste in the biohazard bag for incineration.
- g) For small spills inside the BSC that do not involve the grille area, blot the spill with paper towels and dispose in the biohazard bag. Once removed, use water and paper towels to clean the area as described above, followed by Clidox solution. Dispose off all materials in the biohazard bag for incineration. Change gloves and remove soiled PPE before proceeding. If a STZ spill occurs outside of the hood or involves the grille area of the BSC, immediately leave the room, shut and lock the door. Post a sign indicating "CHEMICAL SPILL" and notify personnel in the immediate area of the potential hazard. Notify Environmental Health and Safety at 806-742-3876 during business hours or 806-742-3328 during non-business hours.

ANIMAL CAGE HANDLING

- a) The animal cage must be clearly labeled with a sign indicating STZ is in use.
- b) Rodents should be housed in microisolator cages.
- c) Animals, cages and bedding are considered hazardous for 48 hours after administration of STZ. The first cage change after each drug administration is not to be performed until at least 48 hours after STZ administration. The alpha-dri bedding is considered contaminated and will be disposed off as incinerated waste and picked up by EHS.
- d) Use of the alpha-dri bedding will help minimize aerosolization of STZ. The 48-hour bedding change should be handled using procedures that minimize the potential for aerosol formation and bedding should only be changed in a BSC to minimize exposure. Personnel performing the cage/bedding change should wear PPE as listed above, including an N95 respirator (in the event a BSC is not available). Standard ABSL 2 husbandry procedures should be followed for the first cage change post STZ injection; however, all STZ waste will be incinerated through EHS rather than autoclaved out of the facility. Reference IACUC SOP 35 for appropriate ABSL 2 husbandry practices
- e) Dirty caging is processed through the cage washer.
- f) Excess feed should be disposed of with the bedding and incinerated through EHS. Wire bar lids and the exterior surface of the water bottles should be sanitized in the cage washer in routine fashion.

ANIMAL HEALTH MONITORING

- A. Animals are observed daily by animal care staff for any evidence of illness or change in behavior.
 - 1. Everyone with access to the animal facility is responsible for immediately informing the facility manager or university veterinarians when an animal becomes ill or a change in behavior is observed.
- B. In the event of suspected illness:
 - 1. Complete the appropriate sick animal care- include the date, the problem observed, and your initials
 - 2. Immediately contact the animal health technician, ACS facility manager or the university veterinarians:

Sydnee Woodman: ACS facility manager
806-834-2872 Office
602-758-0670 Cell

Tiffanie Brooks: ACS/ University veterinarian
806-834-8588 Office
806-239-2120 Cell

Dr. Paul Stonum, ACS Clinical Veterinarian
806-834-7373 Office
660-562-4425 Cell