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

BACKGROUND
Streptozotocin is a probable human carcinogen and experimental teratogen. It accumulates in the liver, kidney and pancreas. After IV or parental 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 recommended 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
DDW
Clidox
Syringe filter (0.22 um)
Acetate Buffer (1mL per 5 mice or 1 rat)
Small red top vacutainers (one /rat or /5mice)
1 mL syringes (one /rat or /5mice plus one)
27 G needles (one /rat or /5mice)
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 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. Aliquot the streptozotocin into the red vacutainers according to weight needed (eg. 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 handlers.
2. Weigh animal and record weight.
3. Follow below for correct dose.

RATS: 55mg/Kg

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\frac{55 \text{ mg}}{1 \text{ Kg}} \times \frac{1 \text{ mL}}{55 \text{ mg}} \times \frac{1 \text{ Kg}}{1000 \text{ g}} = 0.001 \text{ mL/g body weight}
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MICE: 250, 300, or 350 mg/Kg

NUDE 250 mg x 1 mL x 1 Kg = 0.0045 mL/g body weight

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\frac{250 \text{ mg}}{1 \text{ Kg}} \times \frac{1 \text{ mL}}{55 \text{ mg}} \times \frac{1 \text{ Kg}}{1000 \text{ g}}
\]

BALB/C 300 mg x 1 mL x 1 Kg = 0.0055 mL/g body weight

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\frac{300 \text{ mg}}{1 \text{ Kg}} \times \frac{1 \text{ mL}}{55 \text{ mg}} \times \frac{1 \text{ Kg}}{1000 \text{ g}}
\]

C57BL/6 350 mg x 1 mL x 1 Kg = 0.0064 mL/g body weight

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\frac{350 \text{ mg}}{1 \text{ Kg}} \times \frac{1 \text{ mL}}{55 \text{ mg}} \times \frac{1 \text{ Kg}}{1000 \text{ g}}
\]

4. Calculate amount of drug needed.
5. Take 1 mL of buffer and inject into vacutainer with syringe and 18 G needle. Vortex for 30 seconds. (You may reuse this needle and syringe for each tube).
6. Withdraw STZ/buffer solution using new syringe and 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 and dispose of all drug waste into labelled double biohazard bags. Needles go into a regular sharps container.
9. Wipe Down any contaminated surfaces with Clidox.

ANIMAL EXPERIMENTS
a) Prior to initiation of study, arrangements will be made to house rodents in a room.
separate from other rodents. After administration of STZ, rodents will be placed in clean microisolator cages with fresh alpha-dri bedding to prevent aerosolization, a clean wire bare lid, and clean water bottles that are full. Microisolator cages should not be opened during the first 72 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 microisolator cages within the first 72 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 72 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 biohazard sign during the procedure.

d) Animal handlers should wear PPE, including face shield, 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 place inside the BSC for waste materials. A small sharps container will 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 immediately be 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 of waste as 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 of 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 742-3876 during business hours or 742-3328 during non-business hours.

**ANIMAL CAGE HANDLING**

a) The animal housing room door must be clearly labeled with the universal biohazard sign indicating STZ is in use.

b) Rodents should be housed in microisolator cages and the room should not be used for housing of other animals not involved in the protocol.

c) Animals, cages and bedding are considered hazardous for 72 hours after administration of STZ. The first cage change after each drug administration is not to be performed until at least 72 hours after STZ administration. The alpha-dri bedding is considered contaminated and will be disposed of as incinerated waste and picked up by EHS.

d) The 72 hour bedding change should be handled using procedures that minimize the potential for aerosol formation and bedding should only be changed in a Biosafety Cabinet (BSC) or a dump station to minimize exposure. Use of the alpha-dri bedding will help minimize aerosolization of STZ. Personnel making the cage/bedding change should wear PPE as listed above, including a N95 respirator.

e) Dirty caging is processed through the cage washer.

f) Excess feed should be disposed of with the bedding. 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. Record your observations in the ferret’s individual record- include the date, the problem observed, and your initials
2. Immediately contact the 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