

## Streptozocin Injection (low dose).v1

### Procedures:

1. **IMPORTANT: Inform Vivarium supervisor (Valerie Spencer at [vds3f@Virginia.EDU](mailto:vds3f@Virginia.EDU), 434-924-5293)** that you are going to perform STZ injection in mice. Prepare a door label indicating the date, time and dose of the intended injection and hang it on the door of the mouse room.
2. Remove the food from mice (DO NOT remove the water bottle) and **fast the mice for 6 hrs** starting around 9 am.
3. **Prepare Citrate buffer** fresh in the morning by combining 2 part of 0.1 M Sodium citrate with 3 part of 0.1 M Citrate acid to produce 0.1 M Citrate buffer. Adjust the pH to 4.0 by using 1 N NaOH. Filter-sterilize the Citrate buffer and store it in a sterile conical tube on ice.
4. **Weigh the mice** and ear tag them or mark them with an ear puncher.
5. **Add Citrate buffer to Streptozocin (STZ)** (200 µl/mg) in a sterile conical tube in a biosafety hood right before use to a final concentration of 5 mg/ml and keep on ice.
6. **Inject STZ** at 50 mg/kg, i.p. at 3 pm.

Mouse weight (g)	Volume of Injection (ml)
20	0.2
25	0.25
30	0.3
7. **Inject Citrate buffer** for the control group with the same volume calculated according to the body weight.
8. **Add the food back to the cage** after injection each day.
9. **Repeat steps 2-8** for 4 more days.
10. **Measure blood glucose** from the tail vein 72 hrs (3 days) after the last injection. Mice with blood glucose greater than 250 mg/dl are considered successful T1D mice.
11. **It takes 10 weeks before mice develop diabetic cardiomyopathy and heart failure.**

### Reagent and solution preparation:

#### **Streptozocin powder**

Weigh Streptozocin (Sigma 85882) and store at -20°C in aliquots (~1.25 mg/mouse assuming body weight of 25 g. For example, if we have 3 mice weight 25 g, we will need about 3.75 mg of STZ each day) in Eppendorf tubes that are sealed well with cap and parafilm, and clearly labeled with the chemical name and weight.

#### **0.1 M sodium citrate**

Dissolve 1.47 g of sodium citrate tribasic dihydrate (Sigma C0909, MW 294.10) in 50 ml ddH<sub>2</sub>O and store at room temperature.

#### **0.1 M citric acid**

Dissolve 1.05 g of citric acid monohydrate (Sigma C1909, MW 210.14) in 50 ml ddH<sub>2</sub>O and store at room temperature.