

## Streptozocin Injection.v1

### Procedures:

1. **IMPORTANT: Inform Vivarium supervisor** (Laurie Edwards at [lrc8r@Virginia.EDU](mailto:lrc8r@Virginia.EDU) or Alice Kenney at [ask5g@virginia.edu](mailto:ask5g@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 waster bottle) and **fast the mice overnight** starting around 5 pm (~16 hours).
3. **Prepare Citrate buffer** fresh in the morning by combining 20 ml of 0.1 M Sodium citrate with 30 ml 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.
5. **Add Citrate buffer to Streptozocin** (STZ) in a sterile conical tube in a biosafety hood right before use to a final concentration of 20 mg/ml and keep on ice. For example, add 2.5 ml of Citrate buffer to 50 mg of Streptozocin.
6. **Inject STZ** at 200 mg/kg, i.p. (0.1 ml/10 g).
7. **Inject Citrate buffer** at 0.1 ml/10 g for the control groups.
8. **Feed the mice with 10% sucrose** in place of water on the 1<sup>st</sup> day of injection.
9. **Remove the sucrose** and feed the mice with fresh water the next day.
10. **Monitor body weight, blood glucose and ketone bodies** after injection as needed.
11. **Harvest tissue samples** after euthanize the mice with CO<sub>2</sub>.

### Reagent and solution preparation:

#### **Streptozocin powder**

Weigh Streptozocin (Sigma 85882) and store at -20°C in aliquots (25-100 mg) in conical 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.