High-Dose Streptozotocin Induction Protocol (Mouse)

Version: 1
Replaced by version: N/A
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Summary: This protocol is used by DiaComp members to induce diabetes in a number of the animal models developed by the consortium. STZ is toxic to the insulin-producing beta cells of the pancreas and used to induce a diabetes similar to a type I diabetic (Reference). Some reports suggest cellular toxicity outside of the pancreas. STZ also exhibits broad spectrum antibacterial properties and alters the gut microbiota. Please ensure that appropriate controls are included in all studies and complementary models considered (e.g. the $Ins2^{Akita}$ mouse).

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
STZ-Na-Citrate Solution	See below		
TB or insulin syringes w/ needle attached (26-28 ½ gauge)	As many as necessary for the number of mice		
Isoflurane drop jar	Optional		
Isoflurane	Optional		

Protocol:

IMPORTANT: **Mice should fast for four (4) hours prior to STZ induction.** ***The consortium has agreed that a proper fast for the mice is 4-6 hours. Some of the other institutions may fast for 6 hours; we have chosen 4 hours as a proper fasting time.***
The STZ-Na Citrate buffer solution should only be prepared immediately before injection as the drug degrades after 15-20 minutes in the Na-Citrate buffer.

1. Prepare the buffers and solutions as described below. Please note that the STZ-Na-Citrate solution should be prepared immediately prior to injection so as to avoid degradation of the STZ.

2. Mice should be fasted prior to injection; four hours is usually sufficient.
3. Place one mouse in the Isoflurane drop jar, according to your local IACUC anesthesia standards.
4. Remove mouse when breathing has slowed and animal is anesthetized
5. Inject appropriate amount of the STZ solution IP so the final dosage is 1**50 mg/kg mouse**
6. Allow mouse to awaken and place back in cage
7. Repeat procedure for each animal; keep in mind that Isoflurane will need to be re-loaded after every third or fourth animal for proper anesthesia.
8. Each mouse should be given one injection.
9. Supply mice with 10% sucrose water overnight to avoid sudden hypoglycemia post-injection.
10. **Mice should be tested for sufficient levels of hyperglycemia two days after injection and 4 weeks post-injection**. Mice should be severely diabetic.

Reagent Preparation:

Na-Citrate Buffer:

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number
Na Citrate (enzyme grade)	1.47g for 50ml	Fisher Scientific	# BP 327-1
Deionized water (ddH2O)	50ml		
pH meter			

Procedure

- Dissolve 1.47 g of Na Citrate in 50 ml ddH2O
- Test pH with pH meter, adjust buffer to 4.5 pH with monohydrate Na Citrate solution if necessary.

- Buffer should be made fresh with every group of injections
- Place appropriate amount of buffer into a sterile conical tube.

Streptozotocin (STZ):

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number
Streptozotocin		Sigma	# S-0130
Eppendorf tube			
Aluminum foil			

Procedure

Concentration: 22.5mg/ml

- STZ should be stored at –20 C.
- Weigh the appropriate amount of STZ so your final concentration in the Na-Citrate buffer will be 22.5mg/ml; place this into an Eppendorf tube; cover with aluminum foil (light sensitive).

STZ Na-Citrate Solution:

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number
Na-Citrate Buffer from above			
STZ weighed from above			
3 ml 23 gauge syringe/needle			
Empty sterile vial			

Procedure

Concentration: 22.5 mg/ml Dosage: 150 mg/kg mouse

- Do not mix STZ into buffer until ready for injections; the drug degenerates within 15-20 minutes in solution.
- Pour contents of Eppendorf tube (STZ) into the conical (buffer).
- Mix well
- Aspirate the solution using a 3 ml 23 gauge syringe/needle (may need to repeat this several times depending on amount of solution)
- Inject contents into the empty sterile vial
- Vial contains solution for injecting the mice