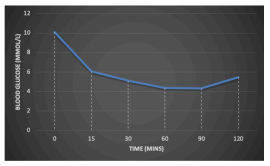
Apr 14, 2020

Insulin Tolerance Test in Mouse



DOI
dx.doi.org/10.17504/protocols.io.wxjffkn

Nancy Smith[1], Mourad Ferdaoussi[1], Haopeng Lin[1], Patrick E Macdonald[1]

[1]University of Alberta

CIRTNR2FIC

Nancy Smith
University of Alberta

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.wxjffkn

External link: http://www.bcell.org

Protocol Citation: Nancy Smith, Mourad Ferdaoussi, Haopeng Lin, Patrick E Macdonald 2020. Insulin Tolerance Test in Mouse. **protocols.io** https://dx.doi.org/10.17504/protocols.io.wxjffkn

Protocol status: Working
We use this protocol and it's working

Created: January 09, 2019

Last Modified: April 14, 2020

Protocol Integer ID: 19147

Keywords: Insulin, tolerance test, ITT, mouse, fasting, high fat diet, IP,

Abstract

This protocol details the insulin tolerance test in mice. This is performed on C57Bl6, transgenic SENP1 KO and ZMIZ1 KO mouse models. Typically glucose tolerance tests (GTTs) are performed at 12 weeks of age, followed by an ITT 2 weeks later. Some mice are put on High Fat Diet and an ITT repeated following 10 weeks on High Fat Diet.
Once the *in vivo* studies are complete, the pancreas or islets are isolated for further *ex vivo* experiments.

Materials

MATERIALS

- Mouse Diet High Fat (60%) **VWR international Ltd** **Catalog #**F3282
- 5LOD Irradiated Pico Lab Rodent Diet **Catalog #**6954
- OneTouch Ultra Blue Blood Glucose Strips **Catalog #**L8041261
- One Touch Ultra 2 Blood Glucose monitoring system **Catalog #**L1540947
- Phosphate Buffered Solution (1X) **Fisher Scientific** **Catalog #**10010-049
- Humulin R (100units/ml) **Catalog #**HI0210
- 1cc Syringes **BD Biosciences** **Catalog #**B309659
- 26G needle **BD Biosciences** **Catalog #**305111

Fasting

1 Fasting begins first thing in the morning (around 9am). Fast mice for 4-6 hours before ITT begins. Transfer mice to clean cage and wire top. Keep water bottles during the fasting period.

Note

- If using High Fat diet (HFD), save food to give back at the end of the ITT.
- Mark tails with a sharpie for easier identification during ITT.

Insulin Solution prep

2 Stock 1: 50ul of 100U/ml Humulin R into 4950ul of cold sterile 1X PBS. Invert solution 3-4 times to mix.
Stock 2: 1ml of Stock 1 into 9ml of cold sterile 1X PBS. Invert solution 3-4 times to mix.

Note

Keep on ice until use

ITT

3 Weigh mouse to obtain body weight for dose calculations.
Calculate bolus dose of insulin (**1-1.2 units/kg**).

Note

Weight of mouse (g) x 10 = ul insulin stock 2 solution

ITT - template spreadsheet.xlsx

4 Prepare syringes using 1cc syringe and 26G needle. Load all syringes with calculated insulin dose and position in front of home cage.

5 Prior to the delivery of the insulin solution, a time “zero” blood glucose level must be measured. Restrain mouse and clean tail with 70% ethanol/gauze. Extract a small amount of blood from

the tail vein onto One Touch Ultra blood glucose strips used with the One Touch Ultra 2 blood glucose monitoring system.

6 Administer the correct dose of Insulin solution using an IP (intraperitoneal) injection method.

1. Restrain the mouse. Then place the mouse is in dorsal recumbency with the head down allowing the viscera to move cranially.
2. Using the bend of the knee and the ventral abdominal midline as landmarks, the needle is inserted half way between the midline and lateral side of the animal at the natural bend of the knee. Insert the needle at a 30 to 45 degree angle into either the left or right lower abdominal quadrant.
3. Prior to injection, aspirate to make sure the needle has not penetrated a blood vessel, the intestines or the urinary bladder. When aspirating the syringe, you should see an air bubble in the hub of the needle, and not any form of fluid.
4. Inject and return mouse to cage.

7 Begin timing from the point of successfully delivering the insulin solution.

8 Test and record the blood glucose level (see step 5) at times 15, 30, 60, 90, and 120 minutes after the initial glucose delivery. Record each value on Insulin Tolerance Test record sheet (template found on step 2). Record condition of animal throughout and after procedure on the Insulin Tolerance Test recored sheet.

9 Return food and environmental enrichments to cages.