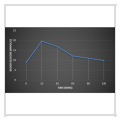

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# IP Glucose Tolerance Test in Mouse

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1 Works for me dx.doi.org/10.17504/protocols.io.wxhffj6

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ABSTRACT

This protocol details the IP glucose tolerance test in mice. This is performed on C57Bl6, transgenic SENP1 KO and ZMIZ1 KO mouse models. Typically GTT's are done at 12 weeks of age. Following the GTT on chow diet, some mice are put on high fat diet for the next 10 weeks. Subsequent glucose tolerance tests are performed following 2 days, 4 weeks and 8 weeks of high fat diet.
Insulin tolerance test can also be performed. Once the *in vivo* studies are complete, the pancreas or islets are isolated for further *ex vivo* experiments.

EXTERNAL LINK

http://www.bcell.org

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Mouse Diet High Fat (60%) | F3282 | VWR international Ltd |
| 5LOD Irradiated Pico Lab Rodent Diet | 6954 | |
| Sterile Glucose (50% dextrose) solution 25g/50ml | 06648(13) | |
| Microvette 100 LH | 20-1282-100 | Sarstedt |
| STELLUX Chemi Rodent Insulin ELISA | 80-INSMR-CH01 | Alpco |
| OneTouch Ultra Blue Blood Glucose Strips | L8041261 | |
| One Touch Ultra 2 Blood Glucose monitoring system | L1540947 | |
| 1cc Syringes | B309659 | BD Biosciences |
| 26G needle | 305111 | BD Biosciences |

## Fasting

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

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



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IPGTT

2 Weigh mouse to obtain body weight for dose calculations.
Calculate bolus dose of glucose (**1g/kg**).

Weight of mouse (g) x 2 = ul sterile glucose (50% dextrose) solution

*If using HFD for 4 weeks or more, use 0.5g/kg (weight of mouse (g) x 1 = ul sterile glucose (50% dextrose) solution)

GTT- template spreadsheet.xlsx

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

4 Prior to the delivery of the sterile glucose (50% dextrose) 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. Blood can be collected into microvette tubes that can be used to extract plasma which can be assayed for plasma insulin (see step 9 for plasma collection).

Microvette tubes are blood collection tubes with a capillary. Tap tube until all the blood is collected in the tube. Place on ice until plasma collection.

5 Administer the calculated dose of sterile glucose (50% dextrose) 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.

6 Begin timing from the point of successfully delivering the sterile glucose (50% dextrose) solution.

7 Test and record the blood glucose level (see step 4) at times 15, 30, 60, 90, and 120 minutes after the initial glucose delivery. Record each value on Glucose Tolerance Test record sheet (template found on Step 2). Record condition of animal throughout and after procedure on Glucose Tolerance Test record sheet.

Extract a small amount of blood from the tail veil at 15, 30, 60 and 120 minutes as well as testing the blood glucose level.
*skip blood collection at 90 minutes to ensure not to exceed 0.007 ml blood/ g body weight.

8 Following experiment, return food and environmental enrichments to cages.



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Plasma collection

9 Place tubes into pre-chilled centrifuge. Centrifguge for 2 mins at 2000 rpm.

**00:02:00**

**4 °C**

10 Remove the capillary-lid and dispose in biohazard bin. Place the base lid on and seal the microvette (click position).

11 Centrifuge at 4°C for 10 mins at 10 000 rpm.

**00:10:00**

**4 °C**

12 Using a pipette, transfer the plasma (top transparent layer) to a clean and labelled tube.

Store plasma samples at -20°C until assayed.

13 Assay for insulin plasma on a STELLUX Chemi Rodent Insulin ELISA kit by ALPCO. See protocol at: https://s3.amazonaws.com/alpco-docs/80/80-INSMR-CH01.pdf





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