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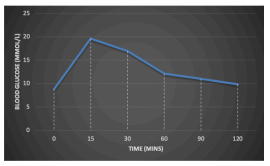
Oral Glucose Tolerance Test in Mouse



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

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Abstract

This protocol details the oral 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.

Materials

MATERIALS

- Mouse Diet High Fat (60%) **VWR International (Avantor)** **Catalog #**F3282
- 5LOD Irradiated Pico Lab Rodent Diet **Catalog #**6954
- Sterile Glucose (50% dextrose) solution 25g/50ml **Catalog #**06648(13)
- Microvette 100 LH **Sarstedt** **Catalog #**20-1282-100
- STELLUX Chemi Rodent Insulin ELISA **Alpco** **Catalog #**80-INSMR-CH01
- OneTouch Ultra Blue Blood Glucose Strips **Catalog #**L8041261
- One Touch Ultra 2 Blood Glucose monitoring system **Catalog #**L1540947
- 1cc Syringes **Becton Dickinson (BD)** **Catalog #**B309659
- 18G gavage needle- curved **Fine Science Tools** **Catalog #**18061-50

Fasting

1 Fasting begins first thing in the morning (around 9am). Fast mice for 4-6 hours before OGTT 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 OGTT.
- mark tails with a sharpie for easier identification during OGTT.

OGTT

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

Note

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 18G oral gavage 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).

Note

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 correct dose of sterile glucose (50% dextrose) solution using the syringe and gavage needle.

1. Restraint of the Mouse, avoiding an excessively tight grip of the mouse, as this will induce a panic response. Ensure that the mouse cannot bite you and cannot move its head.
2. Gravity alone should be used to move the cannula down the esophagus. While gliding the cannula, rotate slightly as the cannula goes past the epiglottis and into the esophagus.The mouse should swallow the cannula, and this can be observed.
3. Inject the fluid slowly.
4. The cannula is then gently removed and the animal is returned to its 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 the Glucose Tolerance Test record sheet.

Note

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 Return food and environmental enrichments to cages.

Plasma collection

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

00:02:00

4 °C

10 Remove the callipary-lid and dispose in biohazard bin. Place the base lid 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.

Note

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