

Insulin tolerance test (ITT)

This protocol may be used to perform ITT on mice.

Keywords: [insulin](#) [blood glucose](#)

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Reagents, supplies, and equipment:

- #21 surgical blades
- 21 ½ G needle
- insulin syringe
- 5 ml syringe
- human insulin 100 IU
- 0.9 % sterile saline
- BD logistic glucometer
- BD glucose test strips
- Clean mouse cages (with normal bedding: dried corn)
- bench paper
- 250 ml plastic beaker
- 70% EtOH

Procedure: Day 1

1. Bring mice to lab around 5:30 PM
2. Disinfect work area with 10% Clorox, then 70% EtOH
3. Prepare work area with balance, clean bench paper, needles, blades, glucometer, and insulin syringes.
4. Allow mice to rest in the lab O/N for ~ 15 hr. Provide food and water as normal.

Procedure: Day 2

1. Fast mice for 6 hr between 9:00 AM - 3:00 PM. (Place mice in a clean cage without food and with H₂O).
2. Weigh mice at 2:30 PM.
3. Calculate the insulin dose for each mouse (based on body weight).
4. Prepare 0.25 IU insulin solution by diluting insulin in sterile saline (and sterile tubes) @ 1:400; mix by vortexing..
5. Dispense the required volume of insulin solution for each mouse into separate 1.5 ml tubes (Volume is calculated as follows:
0.75 IU insulin/kg BW). $\text{Vol } (\mu\text{l}) = 3 \times \text{BW}$
6. Measure blood glucose of the 1st mouse from tail blood by cutting < 5 mm of the tail tip.
7. Perform intraperitoneal injection (i.p.) of insulin. This is time 0' (3:00 PM). Record blood glucose level(s).
8. Continue i.p. injections on subsequent mice, maintaining at least a 1 min interval between animals.
9. Repeat blood glucose measurement at 15', 30', 45', 60', and 90' by reopening the tail wound each time. There is no need to re-cut the tail.
10. Upon completion, provide food to each mouse and return the mice to the mouse colony.

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Attachment

 [itt_worksheet.doc](#) - Added on June 11, 2010 at 11:12 AM by Jill Lindner