



# UC Davis MMPC-Live Protocol

## Intraperitoneal Insulin Tolerance Test

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### Summary:

An intraperitoneal insulin tolerance test or ipITT is designed to determine the sensitivity of insulin-responsive tissues in the rodent. This is determined by measurement of glucose remaining in the circulation over time after a bolus ip insulin injection.

### Reagents and Materials:

<i>Reagent/Material</i>	<i>Vendor</i>	<i>Stock Number</i>
Humalin R	Eli Lilly	R-100
Ultra2 Glucose meter	ONE Touch	
Ultra test strips	ONE Touch/Any Brands	
Sterile 0.9% saline solution	Any	
50% Dextrose	USP	
Sterile 20 ml syringe w. 16 G needle	Any	
Sterile 1 ml syringe w. 25 G 5/8 needle	Any	
Scale	Mettler-Toledo	
Timer	Any	
Stainless steel disposable scalpels	Any	
Gauze & 70% alcohol	Any	
Lab coats/gloves/PPE	Any	
Disinfectant	Nolvasan 10%	
Topical anesthetic cream-optional	Any	

### Protocol:

**Notes:**

- The insulin dose will depend on the level of insulin sensitivity for the mice. Typical doses range from 0.5U/kg to 2.0U/kg of BW. This protocol is written for 0.5U/kg. For a higher dose of insulin, adjust the Insulin Injection Solution concentration accordingly.
- If the concentration of insulin injected is too high for the level of insulin sensitivity, the mice will become severely hypoglycemic (BG < 25mg/dL) and the mice will enter insulin shock (severe lethargy followed by seizure). Since the insulin sensitivity can vary in individual mice, **it is critical to have a glucose solution on hand** to treat mice in insulin shock, by injecting 10 $\mu$ l of 20% glucose/g BW if symptoms are observed.

1. The evening before the ipITT test, weight all mice and fast overnight (16-18h) by transferring mice to clean cages with no food. **NOTE:** Ensure access to drinking water.
2. On the morning of the ipITT, lace the mouse in a duplex cage with mouse of same sex. **NOTE:** no food in cage but access to water is critical. Order cages in the order of ipITT test.
3. Set the test strip in the glucose meter, part of the way. If left in all the way, the meter tends to turn off before blood is administered. **NOTE: Recap the strip container every time after taking a strip. Do not use strips that have been left open to air for extended periods of time.**
4. Clean tail with gauze soaked with 70% alcohol, then dry tail with dry gauze.
5. Score the tip of the tail using a fresh or sterilized scalpel blade, only a millimeter or two is needed.
6. Push test strip all the way into the glucose meter, drop indicator will show up.
7. Milk the tail and discard the first small drop of blood by dabbing on lab mat.
8. A small drop of blood (<5 $\mu$ l) from the tail is placed on the glucose test strip in the glucose meter. Make sure blood fills up the strip and touches the base before the 5 second count down ends to get an accurate reading.
9. Record this baseline glucose fasting level.
10. Inject the mouse intraperitoneally with 10 $\mu$ l/g BW (determined before fasting the mice, in Step 1) of Insulin Injection Solution and note the time-point of injection on the record sheet.
11. The blood glucose levels are measured at 15, 30, 60 and 120 minutes after insulin injection, by placing a small drop of blood on a new test strip and recording the measurements. Start the bleeding again by removing the clot from the first incision; massage the tail if blood flow is inadequate from base to tip. Record results on the record sheet.
12. Ensure that further blood loss from the incision is minimal by briefly applying pressure to the incision after each measurement. At the end of the experiment add food and recovery gel to the cage and make sure water is available to the animals.
13. Monitor the animals carefully to observe any abnormal behavior(s).

**NOTE: change gloves between mice**

## Reagent Preparation:

### *Insulin Injection Solution (see note above regarding final concentration)*

Dilute the insulin stock solution (100 U/ml) with saline to 0.5 U/mL (1/2000 dilution) by adding 10  $\mu$ l stock (100 U/mL) to 20 ml 0.9% (w/v) sterile saline

### *20% Glucose Solution*

Dilute the 2 ml of 50% glucose stock solution with 3 ml 0.9% (w/v) sterile saline