# Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).v1

This is a protocol for collecting mouse blood for assessment of HOMA-IR as an index of insulin sensitivity in mice. The insulin ELISA protocol is adopted from the manufacturer's instruction for Wide Range Assay for the Crystal Chem Ultra-Sensitive Mouse Insulin Elisa Kit (Cat #90080). Calculation of HOMA-IR is adopted from Matthews et al (1985). *Diabetologia* and conversion factor of insulin adopted from Knopp et al (2019). *J Diabetes Sci Technol*.

## Blood Collection

1. Fast mice overnight for 14 hours (remove food from 7:30pm – 9:30 am). *Place mice in sansi-chip (non-corn cob) bedding cages so they do not eat the bedding or old feces.
2. Label microcentrifuge tubes to collect blood (0.5 ml-tubes).
3. Bring mini-table top centrifuge to the mouse room.
4. Clip tail to collect blood for measurement of glucose using a glucose meter.
5. Collect 5-6 drops of blood into a 0.5 ml-microcentrifuge tube and immediately spin down so blood resides in bottom of the tube.
6. Repeat blood collection until you have ~100 µl of blood collected.
7. Set samples in RT for ~4 hours.
8. Spin samples for 15 min at 1500 x g at 4°C.
9. Collect serum (top clear layer) and transfer to a new 0.5 ml microcentrifuge tube.
10. Store samples in -80°C freezer.

## Insulin ELISA

1. Reconstitute Mouse Insulin Standard by dissolving lypholized insulin (2.56 ng/vial) in 100 µl of $dH_2O$ (25.6 ng/ml).
2. Use 50 µL of this standard for serial dilution and store remaining 50 µl in -80°C.
3. Perform a 1:1 serial dilution of this standard (25.6 ng/ml) with 50 µl of sample diluent obtain standards of 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 ng and use 50 µl of sample diluent as 0 ng standard.
4. Pipette 95 µl of diluent buffer into the wells provided in the kit.
5. Pipette 5 ul of sample or standard into the wells provided in the kit and gently mix by pipette.
6. Incubate the plate at 4°C for 2 hours in the cold room. This step will allow insulin to bind to the wall of the wells.
7. Prepare Wash Buffer by mixing 50 ml 20x Wash Buffer Stock with 950 ml of $dH_20$.
8. Prepare Anti-Enzyme Conjugate (anti-insulin antibody conjugated with horse radish peroxidase). immediately prior to use. For every six modules (well strips), mix 3.6 ml Anti-Insulin Enzyme Conjugate Stock with 1.8 mL Enzyme Conjugate Diluent.
9. Aspirate samples from wells.
10. Wash the wells 5 times with 300 µl of Wash Buffer. Decant wash buffer and tap the plate upside down firmly on a paper towel to remove all Wash Buffer.
11. Pipette 100 µl Anti-Enzyme Conjugate into each well.
12. Cover the plate and incubate at RT for 30 min. This allow anti-insulin antibody binds insulin on the wall of the wells.
13. Aspirate liquid from the wells.

14. Wash 7 times with 300 µl of wash buffer. Decant wash buffer and tap firmly on the plate upside down firmly on a paper towel to remove all Wash Buffer.
15. Dispense 100 µl of Enzyme Substrate containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate for horseradish peroxidase into each well with a multichannel pipette.
16. Incubate for 40 min at RT in the dark. ****Do not cover with aluminum foil.
17. Stop the enzyme reaction by adding 100 µl of Enzyme Stop Solution.
18. Measure the absorbance at 450 nm and 630 nm within 30 min using the Orion Plate reader.

## Calculation of HOMA-IR

1. Copy and paste absorbance at 450 nm and 630 nm into a new excel sheet.
2. Subtract absorbances A450 – A630. This difference will be used for plotting the standard curve and calculating insulin values of the samples.
3. Plot the insulin standards of known concentration (x-value) against the measured absorbance difference (y-value) on a graph in excel. This should be a linear curve.
4. Right click graph to Display: "Equation" and "R-squared value" on chart.
5. Calculate sample insulin concentration with the linear equation, i.e. y = bx+a with y = sample absorbance (A450-A630) and x = sample concentration (ng/ml).
6. Solve equation for x. x = (y-a)/b.
7. Convert insulin units from ng/ml to pmol/l by multiplying by 172.18.

$$Conc\left(\frac{ng}{ml}\right) x \frac{1000\ ml}{1\ l} x \frac{1000\ pg}{1\ ng} \frac{1\ mol}{5808\ g\ of\ insulin} x \frac{10^{12}\ pmol}{1\ mol} x \frac{1\ g}{10^{12}\ pg} = \text{pmol/l}$$

8. Convert insulin to µIU/ml by dividing by a conversion factor of 6

**Table 1.**

Summary of Key Insulin Potency Standards and Calculation of Associated Unit Conversion Factors.

| | Molar mass (g/mol) | Potency (IU/mg) | (mg/IU) | (nmol/IU) | Conversion factor $1\frac{\mu IU}{mL} = [\,]\frac{pmol}{L}$ |
|---|---|---|---|---|---|
| Fourth International Standard (1959) | 5808 | 24 | 0.01417 | | 7.174 |
| NIBSC code: 83/500 (1986) | Impure insulin | 26 | 0.0385 | 6.0[a] | 6.622[a] |
| | 5808 | 28.8 | 0.0347 | 6.0 | 6.00 |
| Common (incorrect) conversion factor | 6000 | 24 | 0.01417 | | 6.944 |

[a]These numbers differ as the 1986 standard contains some water and salts.

9. Convert measured blood glucose from mg/dl to mmol/l by multiplying by .0555

$$Blood\ Glucose\ \frac{mg}{dl}\ x\ \frac{1\ g}{1000\ mg}\ x\ \frac{1000\ mmol}{1\ mol}\ x\ \frac{10\ dl}{1\ l}\ x\ \frac{1\ mol}{180.16\ g} = \frac{mmol}{l}$$

10. Calculate HOMA-IR. Multiple insulin (µIU/ml) by glucose (mmol/l) and divide by 22.5

$$\frac{insulin\frac{uIU}{ml}\ x\ blood\ glucose\frac{\text{mmol}}{\text{l}}}{22.5} = \text{HOMA-IR}$$