



# Hyperglycemic clamp

Version: 1

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## Summary:

Hyperglycemic clamp is used to assess insulin secretory capacity in conscious mice in response to hyperglycemia. Plasma insulin and C-Peptide concentrations are measured at various times during the 2-hr clamp period.

## Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Infusion Pumps	Harvard Apparatus	PY8 70-2208
Stand	Fisher Scientific	14-670A
Dual channel swivel	Instech Solomon	375/D/22QM
3- and 4-way stainless steel connectors	Ziggy's Tubes and Wires	HSCY-25 or HSC4-25
Microrenthane tubing (0.033" OD)	Braintree Scientific	MRE-033
Glucose meter and strips	ACCU-CHEK aviva	
Blunt needle with luer hub	Ziggy's Tubes and Wires	LHN-E011041 25ga x 0.5"
Wire, stainless steel	Ziggy's Tubes and Wires	W020304V-1
Clamp, extension	Fisher Scientific	05-769-7Q
Connector, hook	Fisher Scientific	14-666-18Q
50% dextrose		

## Protocol:

1. Surgical catheterization of the carotid artery and jugular vein in mice at least 5 days prior to the day of the study (refer to protocol for Surgical Catheterization of the Carotid Artery and Jugular Vein).
2. Weigh mouse and start fast (suggested starting time between 7:00 and 8:00 AM) by placing mouse in a plastic container with fresh bedding.

3. Mouse is hooked up to the swivel 4 hours into fasting and basal blood/plasma samples are collected 15 and 5 mins before the start of the clamp (refer to protocol for Hyperinsulinemic-Euglycemic Clamp for detailed set-up and connections).
4. After a total of 5 hr fast, variable infusion of 50% glucose starts. Arterial glucose is increased and maintained at 250-300 mg/dL.
5. Donor blood is infused to jugular vein catheter throughout the study to prevent a fall of hematocrit.
6. Plasma insulin and C-Peptide concentrations are measured at times as described in the following study sheet.
7. At the end of the study, mouse is anesthetized and tissues of interest are harvested and frozen in liquid nitrogen.

TIME (min)	SAMPLE (µl)	GLUCOSE (mg/dl)	Time of infusion change	Glucose infusion rate		HCT	comments
				(µl/min)	mg/kg/min		
-60	<b>Place mouse in tub for acclimation</b>						
-15	50 (G,I)						
-5	100(G,I,C)					*	Start donor blood
0	<b>Variable Glucose Infusion (Clamp to ~300 mg/dl) Donor Blood :7ul/min</b>						
5	50 (G,I)						
10	50 (G,I)						
15	100 (G,I,C)						
20	50 (G,I)						
30	5 (G)						
40	50 (G,I)						
50	5 (G)						
60	50 (G,I)						
70	5 (G)						
80	50 (G,I)						
90	5 (G)						
100	100 (G,I,C)						
110	5 (G)					*	
120	100 (G,I,C)						

I: sample for plasma insulin concentration (25 ul plasma)  
 C: sample for C-peptide at t = -5, 15, 100, and 120 (50 ul blood)

### Reagent Preparation:

[Reagent 1](#)

## Reagent 1: Donor Blood

1. Collect ~ 1 ml of blood from donor mouse in 0.5 ml EDTA tubes.
2. Centrifuge blood (1 min at 16,000 g) and save plasma for preparation of insulin (see below).
3. Resuspend red blood cells (RBC) with heparinized saline (10U/mL).
4. Centrifuge (1 min at 16,000 g), discard supernatant, and resuspend RBC with an equal volume of heparinized saline. Transfer resuspended RBC (donor blood) to a 1.5 ml tube