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Working

## Vandy - Hyperglycemic clamp [↗](#)

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### ABSTRACT

#### Summary:

Mice with catheters implanted in the jugular vein (infusions) and carotid artery (sampling) are used for this procedure. The 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.

### EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=240&docType=Protocol>

### MATERIALS

NAME	CATALOG #	VENDOR
Infusion Pumps	PY8 70-2208	Harvard Apparatus
Stand	14-670A	Fisher Scientific
Dual channel swivel	375/D/22QM	Instech Solomon
3- and 4-way stainless steel connectors	HSCY-25 or HSC425	Ziggy's Tubes and Wires
Microrenathane tubing (0.033" OD)	MRE-033	Braintree Scientific
Glucose meter and strips	View	ACCU-CHEK aviva
Blunt needle with luer hub	LHN-E011041 25ga x 0.5"	Ziggy's Tubes and Wires
Wire stainless steel	W020304V-1	Ziggy's Tubes and Wires
Clamp extension	05-769-7Q	Fisher Scientific
Connector hook	14-666-18Q	Fisher Scientific
50% dextrose		

### MATERIALS TEXT

#### Reagent Preparation:

##### 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

#### Note:

- 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 ( $\mu$ l)	GLUCOSE (mg/dl)	Time of infusion change	Glucose infusion rate		HCT	comments
				( $\mu$ l/min)	mg/kg/min		
-60	Place mouse in tub for acclimation						
-15	50 (G,I)						
-5	100(G,I,C)					*	Start donor blood
0	Variable Glucose Infusion (Clamp to ~300 mg/dl) Donor Blood :7ul/min						
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  $\mu$ l plasma)  
 C: sample for C-peptide at t = -5,15,100, and 120 (50  $\mu$ l blood)

