



Post-Clamp Anesthesia of mice

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Summary:

After the last sample is taken for the clamp, the mouse is anesthetized in order to harvest tissues. It is very important that the mouse remain alive during tissue harvesting for future signaling or metabolite analysis. The mouse is therefore given a sub-lethal dose of pentobarbital to induce anesthesia (~70mg/kg pi or 35mg/kg iv).

Reagents and Materials:

Reagent/Material
Pentobarbital/ Nembutal® at 50mg/mL
Blunted needle
Sterile Saline solution

Protocol:

1. Draw up the desired volume of diluted Pentobarb (5mg/ml in saline) in a blunted syringe.
 - To give 35mg/kg, multiply body weight (grams) by 7, to get the volume to inject in uL.
 - Pentobarb (uL) = BW (g) * 7
2. Steadily inject the pentobarbital in the jugular line of the catheterized mouse.
3. If the mouse is not completely anesthetized when taking the tissues, inject more by 50uL increments.

Concentration:	5 mg/ml							
Pentobarb mg/kg	BW mouse (g)							
	20	25	30	35	40	45	50	55
70	280	350	420	490	560	630	700	770
100	400	500	600	700	800	900	1000	1100

Reagent Preparation:

Reagent 1: Diluted Pentobarbital 5 mg/mL in saline from stock Pentobarbital (Nembutal®, 50mg/mL)

1. For a 30mL Saline bottle: Inject 3.3mL of Stock Pentobarbital (50mg/ml), mix.
2. For a 10mL Saline bottle: Inject 1.1mL of Stock Pentobarbital, mix.