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🌐 Protocol for Mouse IP Anesthesia (Ketamine-Xylazine) V.2

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Human Islet Research Ne…

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the constitution of our anesthesia cocktail that we use for sedating our mice when performing surgeries. The surgeries we typically perform are thymectomies and implantation of material under the kidney capsule (through open abdomen or minimally invasive approaches).

> **Note**
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> The changes of this protocol was made by Austin Chen on Markus Holzl original protocol.

> **Note**
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Materials

Materials:

Anesthesia is a mixture of ketamine and xylazine in 1x PBS.
- ·**Ketamine:** Ketathesia (100 mg/mL; cat: 056344; Henry Schein)
- ·**Xylazine:** Xylazine sterile solution (20 mg/mL; Columbia ICM)
- ·**1x PBS:** Phosphate Buffered Solution (1X) (**cat: BW17516F24; Lonza**)
- ·**0.22 um filter:** Syringe Filter Unit, 0.22 μm, polyethersulfone (**Millex-GP; cat: SLGPR33RS**)

Preparation:

Ketamine stock: 100 mg/mL

Xylazine stock: 20 mg/mL

Desired ketamine concentration: 0.08 mg/gram of mouse

Desired xylazine concentration: 0.012 mg/gram of mouse
- We will make anesthesia solution to dose 🧪 10 μL anesthesia/gram of mouse for IP injections

For 10 mL of anesthesia:
1. 🧪 0.8 mL Ketamine (100 mg/mL) + 🧪 0.6 mL Xylazine (20 mg/mL) + 🧪 8.6 mL 1x PBS
2. Filter solution with 0.22 um Millex-GP filter

Calculations:
- Ketamine: 0.8 mL * 100 mg/mL in 10 mL → 8 mg/mL Ketamine → 0.08 mg/10 uL
- Xylazine 0.6 mL * 20 mg/mL in 10 mL → 1.2 mg/mL Xylazine → 0.012 mg/10 uL

For Intraperitoneal (IP) Approach:

1   Use 10 uL/gram of anesthesia

Comments:

1. It takes time until the mice fall asleep

2. Very often, mice do not fall asleep but move around like zombies. This requires another small dose of IP ketamine-xylazine injection or a small dose of IV injection

a. When doing IP injection again, we have injected IP with a volume of about 1/3 of original volume

b. For IV approach after IP, it takes very little anesthesia for them to fall asleep. We usually use around 10-20 uL. Inject this slowly into the mouse. The mouse should fall asleep rapidly. It is very easy to overdose with IV injection so be careful.