**rotocol for Mouse Imaging**

**# Inject Ketamine based on body weight.Eg 21g then 210 uL**

**Inject Luciferin-std 150uL/mice of 20g**

* **Ketamine preparation 2ml Ketamine+1ml Xylazine+ 17 ml WFI**
* **Luciferin- see the box 15mg/ml 1 bottle powder-> add 65.7ml ultrapure water**

1. Prepare all items: the luciferin (from -20C fridge) (inject how much?), needle, Ketamine.
2. On Computer
3. Must clean the bench thoroughly.
4. Put the black separator (to avoid cross signal) on the board.

|  |  |  |
| --- | --- | --- |
| **1** | **2** | **3** |
| **4** | **5** | **6** |

1. **Stick the wipe on the handle (because NO GLOVE on it)**

**#BLOOD paper on Irradiation vs Non-Irradiation**

*-If cell line->irradiate-> chemo too strong will die also*

1. Weigh the mice and inject the ketamine (Ketamine 100mg/kg +Xylazine 10mg/kg) (IP)- surgical anaesthesia dose. Inject on the right side of the mice.

#Duration of onset 20-30mins

#Sleep time -60-120 mins

1. Swab injection site. Inject 1 by 1.
2. Inject Luciferin (check concentration on box) (which is a substrate). (IP). Inject on the left side of the mice. \*Withdraw 450uL one shot of Luciferin (for 3 mice) into one syringe.
3. After inject luciferin (150uL already in excess-more than enough) -> wait 10 mins for luciferin react.
4. Put those injected luciferin mice on the cover first then only all arrange on the black board.
5. Arrange the mice inside the machine. *Follow the laser light*. Inside the line -> Run machine

* ***Count from last mice only click ’start imaging’***

1. After finish, put back to the cage.
2. Must put WIPE on the bedding as floor mat, otherwise mice will inhale the dust during aneasthenia. Arrange side by side to get warm.
3. If one person, do 2 cages (weigh-> inject-> image)

**Computer operation**

Initialize-> Wast?-> Sequence Set up-> Open file icon-> Data (D)-User-Nelson Ng-> Imaging setup New(file)

After Loading-> Acquire Sequence-> Rename labels

**Protocol for Mouse Imaging Analysis**

**General**

1. Click ‘Initialize’ for *1st User*
2. Binning: Use medium

|  |  |
| --- | --- |
|  |  |
|  |  |

1. Larger aperture, > light captured
2. Emission filter [OPEN]
3. Click [ACQUIRE]
4. Use setting 2e5 & 2e7

**Quantification Flux**

1. Open the txt file format -> change the ‘Count” to ‘Photon” on upper left before draw the rectangle for ROI.
2. ROI Tool ‘Dropdown button’
3. Click same shape rectangle or circle
4. Copy and paste to get the same size for each mouse
5. Click [View] -> click [ROI Measurement]
6. Choose the unit [Photon Flux] \*photon per second
7. Select the info (amount flux) and copy -> export-> open excel and paste it.

1.92e5

4.87e6

7.50e5

1.51e7