Quick Start Guide to Acquiring Images on IVIS 200

Purpose: To prevent cross contamination of mice from different animal housing areas during use of the shared imaging equipment. Mice can carry viruses, parasites, and bacteria that may be shed in the environment during routine handling and procedures. Surfaces exposed to infected mice will easily contaminate other animals if they are exposed to the same contaminated surfaces.

1. **PREPARATION OF IMAGING ROOM**
   - **Wrap cages in clean, disposable drape material for transport from animal housing room to the imaging room (1-639 BSB).**
   - **Wipe away all debris from surfaces of the machine and table tops in the procedure room that may come into contact with the mice or their caging.**
   - **Use saturated lint-free wipe with Clidox-s to clean the internal surfaces of the chamber and the Imaging Platform using a gentle circular motion, and then door handle.**
     AVOID CELANING UPPER CEILING.
   - **Rewash the cleaned surfaces using a lint-free wipe saturated with ddH2O.**

     Do Not Allow water to puddle or remain on the surfaces.

2. **Start Living Image 2.5® software program**
3. **Click the Initialize IVIS system button and then wait till Temperature Status box shows GREEN**
4. **XGI-8 Gas Anesthesia System setting:**
   - **Turn ON the "EVACUATION PUMP"**
   - **Turn ON the supply Oxygen**
   - **Turn ON the gas flow to the XGI-8 using the green Oxygen handle**
   - **Set the Vaporizer value to 2.0 to 2.5 when you needed**
   - **Turn ON the toggle valve entitled "IVIS Flow on/off" on the front panel.**
5. **PREPARATION OF IMAGING EQUIPMENT**
   - **Place the provided black mat (paper for Luminescent or plastic for Fluorescence) on the Imaging Platform where the sample will be placed.**
   - **Place a clean nose cone(s) into the anesthetic outlet(s). Clean cones can be found in the container labeled "Clean Nose Cones" on the table.**
   - **Place sample on the black mat with their nose in the anesthetic cone.**
   - **Remove gloves once mice are in the imaging machine. DO NOT touch any computer equipment with gloved or bare hands that have previously contacted the animal.**
   - **Select the desired Field of View from the pull down menu on the left side of the control panel.**
   - **CLOSE the DOOR. NEVER OPEN DOOR WHEN THE DOOR RED LIGHT IS ON!**

6. Living Image 2.5® Software Program Setting:

- **Enter the approximate (1.5 cm) Subject Height in the lower left entry box.**
- **Click the Acquire continuous photos button to check the subject position.**
- **Check the Overlay box in the control panel and set the Exposure Time desired for the luminescent image. If unsure of what exposure time to use, it is best to start with short times (~ 10 sec) and increase as necessary.**
- **Click the Acquire button on the control panel.**
- **After the exposure is complete, the overlaid image is displayed. Edit the information in the Change Information window and click DONE.**
- **Confirm that the signal of interest is above the noise level (recommend > 100 counts and below CCD saturation (65535 counts). If the signal level is unacceptable, adjust Exposure Time or Binning and repeat the image acquisition.**
- **Under the Living Image menu item, choose Save Living Image Data to save the displayed data. If desirable, adjust the display with Max Bar, Min Bar, Bright, and /or Gamma controls in the top of the image window.**

7. POST IMAGING

- **Place clean gloves back onto hands before touching the animal again after imaging procedures are complete.**
- **Replace animal in cage and rewrap cage in clean drape to be brought back to the animal housing room.**
- **Throw away black paper mat or disinfect black plastic mat with Clidox-s.**
- **Place used nose cones into the "Dirty Nose Cones" container to be disinfected by staffs.**

8. Repeat Clidox disinfection procedures stated above (1)

9. Record time usage on record book.

10. Please call Jian, Tom or Randy if you have any question on this system.

FLUORESCENCE OPTION ON IVIS SYSTEM

1. **Select Fluorescent in IVIS 200 System Control window;**
2. **EXCITATION FILTER: It is very important to always match filters;**
3. **FILTER LOCK: Locks the excitation and emission filters together;**
4. **FLURO LAMP LEVEL: allows the user to set the fluorescence lamp emission level : OFF**

   LOW—Low emission is approximately 18% of High

   HIGH—It is recommended to always use High if possible. If a fluorescent image is too bright, then use other controls such as f/stop, Binning, or Exposure Time to reduce the signal.

   INSPECT—turns the fluorescent lamp on manually, allowing the user to inspect the action inside the IVIS Imaging chamber.

NOTE: The f/stop setting defaults to f/2 for fluorescence imaging. While this is the recommended starting point, the f/stop may be adjusted if necessary.

RECONSTITUTION AND USE OF D-LUCIFERIN FIREFLY POTASSIUM SALT (CAT# XR-1001)

Preparation of Luciferin for In Vitro Bioluminescent Assays

Materials needed:

D-Luciferin Firefly, potassium salt, 1.0 g /vial (Xenogen Catalog # XR-1001)

Sterile water

Complete media

Procedure:

1. Prepare a 200X luciferin stock solution (30 mg/ml) in sterile water. Mix gently by inversion until luciferin is completely dissolved. Use immediately, or aliquot and freeze at -20°C for future use.
   ** Note: One can either reconstitute the entire 1.0 g of D-Luciferin in 33.3 ml of sterile water to make the 30 mg/ml (200x) stock solution, or reconstitute the quantity of D-Luciferin necessary for an individual experiment.*
2. Prepare a 150 ug/ml working solution of D-Luciferin in pre-warmed tissue culture medium. Quick thaw 200X stock solution of luciferin and dilute 1:200 in complete media (150 ug/ml final).
3. Aspirate media from cultured cells.
4. Add 1x luciferin solution to cells just prior to imaging.
   ** Note: Incubating the cells for a short time at 37^{o}C before imaging can increase the signal.*

Preparation of Luciferin for In Vivo Bioluminescent Assays

Materials needed:

D-Luciferin, Firefly, potassium salt, 1.0 g/vial (Xenogen Catalog #XR-1001)

DPBS, w/o Mg^{2+} and Ca^{2+}

Syringe filter, 0.2 um

Procedure:

1. Prepare a fresh stock solution of luciferin at 15mg/ml in DPBS. Filter sterilize through a 0.2 um filter.
2. Inject 10 ul/g of body weight. Each mouse should receive 150 mg luciferin/kg body weight. (e.g. For a 10 g mouse, inject 100 ul to deliver 1.5 mg of luciferin.)
3. Inject the luciferin intra-peritoneally (i.p.) 10-15 minutes before imaging*.
   * A luciferin kinetic study should be performed for each animal model to determine peak signal time.