Preparation for Bioluminescent Imaging

- Select a Stable Cell line (Screen for maximum Luciferase Expression)
- Cell line injection or tumor Implantation[1]
- Preparation of Luciferin solution[2]
- Preparation of Anesthesia Agent[3]
- Remove hair for efficient signal transmission if necessary[4]

Bioluminescent Imaging

Injection of Anesthetic[5] and Luciferin

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Wait for Dispersion of Luciferin
(Determining Luciferin Kinetic Curve for Your Model[6] if necessary)

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Acquire Image with IVIS system (Control Exposure Time, Binning, f/stop If necessary)[7]

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Check for Bioluminescent signals from primary and metastatic sites[8]

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Region Measurement and Analysis

1. Luciferase Transfected cells can be transplanted or injected subcutaneously, intraperitoneally, intravenously or through any other route. .

2. Prepare a fresh stock solution of Luciferin at 15mg/ml in DPBS. Filter sterilize through a 0.2 µm filter. Inject 10 µl/g of body weight. Each mouse should receive 150 mg Luciferin/kg body weight

Brand	Cat number
Caliper	122796
Invitrogen	L2916
GoldBio	LUCK
Promega	E1605

3.

Anesthetic agent	Preparation
Isoflurane Gas	Nose cone apparatus / manifold Activated charcoal evacuation filters
Ketamine/ Xylazine	4:1 mixture of Ketamine (100mg/ml) and Xylazine (20mg/ml).
Pentobarbital	
Chloral hydrate	

4. Removal of hair can be done by shaver or applying Rasera Veet Hair Removal gel cream.

5. To Inject intraperitoneally, restrain the mice with head of animal pointing down. Needle should be bevel-side up and slightly angled when entering the abdominal cavity. Penetrate just through abdominal wall (about 4-5mm). The tip of the needle should just penetrate the abdominal wall of the animal.

6. Take consecutive images with IVIS 100 every 2 minutes up to 60 minutes to generate a kinetic curve for Luciferin signal expression of your model. Choose the best time point to image thereafter. Most of models show maximum signal intensity at 15-30 minutes after Luciferin injection.

7. Please Refer to Standard Operation Protocol – Xenogen IVIS 100

8. Unsuccessful injections will be obvious since the bioluminescent signal will be limited to the Injection Site. Mice sometimes show metastatic signals in liver, kidney, fat, and other tissues

Preparation for Fluorescent Imaging

- Choose a suitable Fluorescent Protein[1] or Fluorescent Staining Reagent Stable Cell line
- Select Maximum Fluorescent staining or expression cell for injection or transplant
- Remove hair to reduce autofluorescent background if necessary[2]
- Feed with Alfalfa-free, or Purified Diet[3] if necessary
- Preparation of Anesthesia Agent[4]

Fluorescent Imaging

Intraperitoneal (i.p.) Injection of Anesthetic

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Acquire Image with Maestro system (Adjust Exposure Time, Binning, Excitation/Detection Spectrum If necessary)[5]

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Spectrum Identification and Spectrum Unmixing

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Display Adjustment, Region Measurement and Analysis

1. Tissue scatters and absorbs light. The heme quench light of wavelengths less than 600nm, while water absorption quenches wavelengths above 900nm. Therefore, preferably choose a fluorophore with emission wavelength of >600nm and <900nm.

2. Endogenous molecule like elastin, collagen, tryptophan, NADH, porphyrins, and flavins present in animal tissue and particularly in hair and skin strongly autofluoresce upon excitation in the wavelengths <600nm. All these autofluorescent are non-specifically excited by the excitation light source and give a strong background during imaging, Removal of hair can be done by shaver or applying Rasera Veet Hair Removal gel cream.

3. Alfalfa and chlorophyll in routine mouse diet show are strongly fluorescent in the digestive tract between 650 and 750nm. It is recommended to feed the animals on an alfalfa free diet for 2 weeks prior to fluorescence imaging.

4.

Anesthetic agent	Preparation
Isoflurane Gas	Nose cone apparatus / manifold Activated charcoal evacuation filters
Ketamine/ Xylazine	4:1 mixture of Ketamine (100mg/ml) and Xylazine (20mg/ml).
Pentobarbital	
Chloral hydrate	

5. Please Refer to Standard Operation Protocol – Maestro 2