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IVIS 200 Protocol for in vivo bioluminescence assay.docx

## Protocol for in vivo bioluminescence assay

### Materials needed:

- 1D-Luciferin, Firefly, potassium salt, 1.0 g/vial Caliper Life Sciences Cat. No. #119222, (Merkel Technologies, agent in Israel)
- Dulbecco's Phosphate Buffered Saline (DPBS), w/o Ca<sup>++</sup> & Mg<sup>++</sup> Syringe

### Procedure:

1. Prepare a luciferin stock solution (15 mg/ml) in sterile DPBS w/o Ca<sup>++</sup> & Mg<sup>++</sup>. Mix gently by inversion until luciferin is completely dissolved. Use immediately, or sterile filter (0.2 µm) and freeze aliquots at -20°C for future use.

**Note:** One can either reconstitute the entire 1.0 g of D-Luciferin in 66.6 ml of sterile DPBS w/o Ca<sup>++</sup> & Mg<sup>++</sup> to make the 15 mg/ml stock solution, or reconstitute the quantity of D-Luciferin necessary for an individual experiment.

2. Inject 10µl/g (150mg luciferin/kg) of body weight (e.g., for a 10g mouse, inject 100µl to deliver 1.5 mg of 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.

3. Set isoflurane vaporizer to appropriate levels to knock mice down
4. Restrain mouse and tilt its head towards the ground to force the organs away from injection site
5. Using a 1ml syringe with a 27.5G needle, inject luciferin stock i.p.
6. Put the mouse into the induction box and allow luciferin to distribute for 5 minutes\*

**Note:** 5 min works for most applications; this is a good starting point. It is generally a good idea however to perform an initial experiment in which a 30sec image is taken at 1min, 3min, 5min, 10min, 15min, and 20min. This allows you to identify the proper time for luciferin distribution for your application.

7. Place mouse into imaging chamber and position into the manifold
8. Be sure that the anesthesia lever on the side of the IVIS is turned on
9. Press the "ACQUIRE" button in the camera control panel interface