UF | ICBR Cytometry
University of Florida, Interdisciplinary Center for Biotechnology Research
(https://biotech.ufl.edu)

CTAC ICBR SOP: Using the IVIS Spectrum to Determine a Luciferin Kinetic Curve for your Model

Materials Required:

D-Luciferin, Firefly, potassium salt, 1.0 g/vial

DPBS, w/o Mg2+ and Ca2+

Syringe filter, 0.2 mm

25 gauge hypodermic needle, usually used with 1 cc syringe

Purpose:

To inform the user of the process necessary for determining a luciferin kinetic curve for the model used / experiment..

Procedure methodology description:

Mice are injected by an intraperitoneal route with a luciferin solution (15 mg/ml or 30 mg/kg, in PBS, dose of 150 mg/kg) that is allowed to distribute in awake animals for about 5-15 minutes. This can be variable dependent on source / vendor.

Position of animal

Manually restrained, dorsal recumbency (abdomen side up), with cranial (head) end of animal pointed down.

Intraperitoneal (i.p.) Injection of Luciferin

Preferred site: Animal’s lower left abdominal quadrant.

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’s left lower abdominal quadrant.

Needle size: 25 gauge, usually used with 1 cc syringe

Volume: 100 ul of luciferin (15 mg/ml stock) per 10 grams of mouse body weight.

Note: 1 ml i.p. injection of a nonirritating solution is easily tolerated.

Anesthesia

The mice are placed into a clear plexiglass anesthesia box (2.5-3.5% isofluorane) that allows unimpeded visual monitoring of the animals; e.g. one can easily determine if the animals are breathing. The tube that supplies the anesthesia to the box is split so that the same concentration of anesthesia is plumbed to the anesthesia manifold located inside the imaging chamber.

After the mice are fully anesthetized, they are transferred from the box to the nose cones attached to the manifold in the imaging chamber, the door is closed, and the “Acquire” button (part of the Living Image® program) on the computer screen is activated. The imaging time is between one to five minutes per side (dorsal/ventral), depending on the experiment. When the mice are turned from dorsal to ventral (or vice versa), they can be visibly observed for any signs of distress or changes in vitality. The mice are again imaged (maximum five minutes), and the procedure is complete. The mice are returned to their cages where they awake quickly.

Generating a Kinetic Curve

To generate a kinetic curve for luciferase activity in your model:

1. Inject luciferin i.p. as described. It is preferable to inject into awake animals. If you need to sedate the mice before injection, this may be done, but it may slightly extend the kinetics (peak luciferase expression time).
2. Wait three minutes, then sedate by your method of choice, gas or injectable anesthesia.
3. Place sedated animals in imaging chamber and take the first image approximately five minutes after the luciferin injection.
4. Continue to take images every 5-10 minutes (or more frequently) up to about 40 minutes to generate a kinetic curve for luciferin expression in your model.

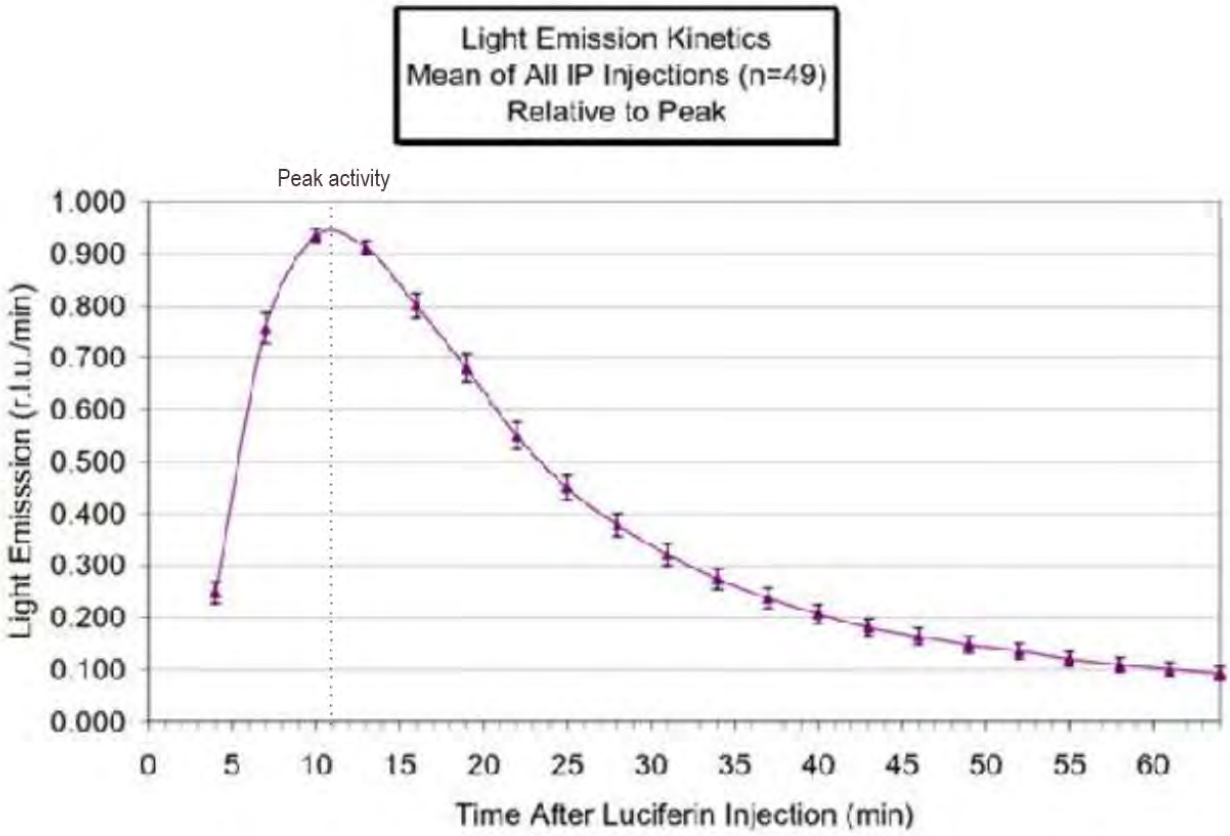


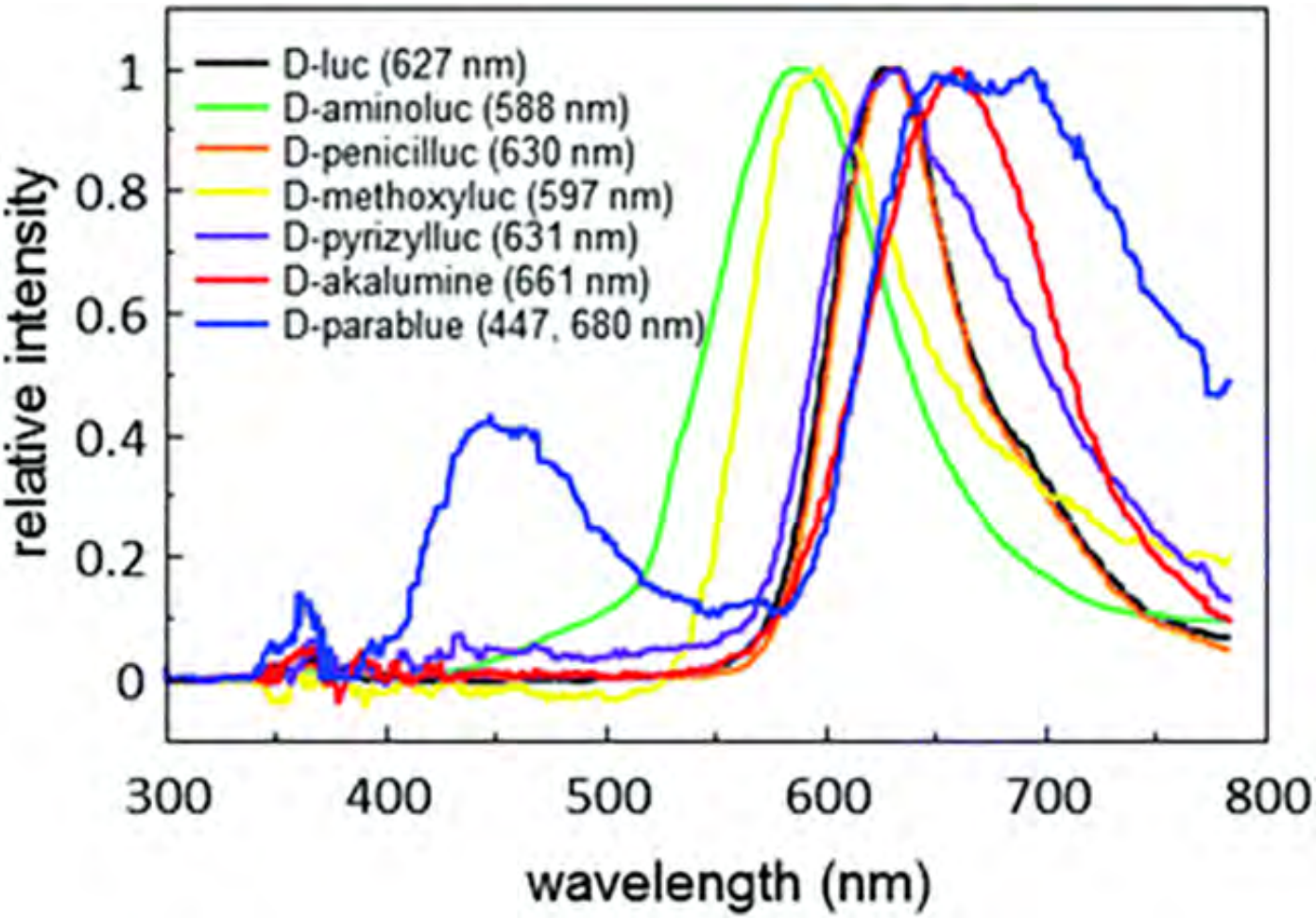
Figure 3.8 – i.p. luciferin injection
Integrated light relative to the peak of the kinetic curves

Credit: R.A. Bollinger. "Evaluation of the Light Emission Kinetics in Luciferin/Luciferase-Based In Vivo Bioluminescence Imaging for Guidance in the Development of Small Animal Imaging Study Design."

With 2% isoflurane gas anesthesia healthy animals can safely be sedated in the IVIS® for well in excess of 45 minutes. **Note**: for all but brief imaging (<5 min) ophthalmic ointment is required to prevent ocular dessication.

5. Once you have established your curve you can choose the best time point to image at thereafter. Most of the models are imaged at 10-20 minutes after luciferin injection.

Simple forms of D-luciferin typically achieve peak bioluminescence at around 10-12 minutes after injection of a subject. However, there are many commercial forms of luciferase product, with a diverse complement of associated chemical groupings attached by way of modification. These alter the peak bioluminescence activity, and the peak may not occur until as late as 30 minutes for some modified version and specific transgenic models.



As can be seen below, the pharmacokinetics also vary in terms of peak activity and biochemical decay dependent upon delivery route.

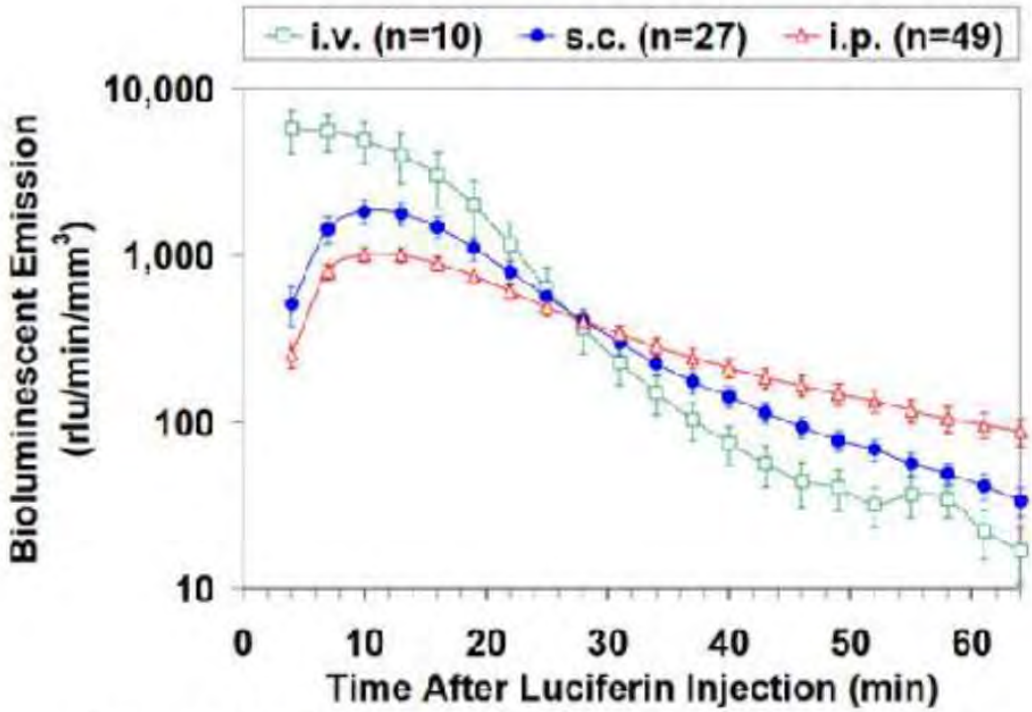


Figure 3.43 – Bioluminescent Light Emission Kinetic Profiles for Various Luciferin Injection Routes (Vol 2) - BLI emission decays exponentially for each injection route, but at very different rates. After 43 minutes, the BLI emission following i.v. injection approaches the level of background noise which effects quantification.

Credit: R.A. Bollinger. "Evaluation of the Light Emission Kinetics in Luciferin/Luciferase-Based In Vivo Bioluminescence Imaging for Guidance in the Development of Small Animal Imaging Study Design."