STANDARD OPERATING PROCEDURE

*In Vivo* Imaging Reagents

# Determining the Luciferin Kinetic Curve for Your Model

Although bio distribution of injected luciferin is rapid, the kinetics of signal production can vary as they may be tissue dependent. Therefore, we strongly recommend that you follow the procedure below to determine the optimal time after luciferin injection in which to image your model.

This procedure should be performed for each new animal model you image.

## Generating a Kinetic Curve for Luciferase Activity in Your Model

1. Inject Luciferin into animal by an intraperitoneal (i.p.) route with a solution of 15 mg/ml or 30 mg/ml, dissolved in PBS, for a working dose of 150 mg/kg.*

2. Wait three minutes, and sedate the animal by your method of choice; gas or injectable anesthesia. (2% isoflurane gas anesthesia allows healthy animals to be safely sedated in the IVIS for 45 minutes.)

3. Place sedated animal in imaging chamber and take the first image, which will now be approximately five minutes after the Luciferin injection.

4. Continue to take images every 5-10 minutes up to approximately 40 minutes. This will indicate the kinetic curve for Luciferin uptake in your model.

5. Once you have established your curve, you can follow the Imaging Procedure to the right, incorporating the best time point in which to image thereafter. (10-20 minutes after Luciferin injection is ideal for most models.)

6. Check the in vitro bioluminescence using the IVIS imaging system every 10 min, up to 40 min, to determine the kinetic curve and find the peak imaging time point for each cell type.

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## Imaging Procedure using the PerkinElmer IVIS® Preclinical *In Vivo* Imaging System

1. Inject Luciferin into animal i.p. with a solution of 15 mg/ml or 30 mg/ml, dissolved in PBS, for a working dose of 150 mg/kg.*

2. Allow to distribute in animal for best amount of time as determined by your kinetic curve.

3. Place mouse into a clear Plexiglas anesthesia box (2.5-3.5% isofluorane) that allows unimpeded visual monitoring of the animal; e.g. one can easily determine if the animal is breathing.

   The tube that supplies the anesthesia to the box is split so that the same concentration of anesthesia is delivered to the anesthesia manifold located inside the imaging chamber.

4. Once the anesthesia has taken full effect, transfer the mouse from the box to the nose cone attached to the manifold inside the imaging chamber. Close the door, and click the “Acquire” button in the Living Image program on the computer screen.

Imaging time is between 1 and 5 minutes per position (dorsal/ventral), depending on the experiment, with 5 minutes per position being the maximum.

When turning the mouse from dorsal to ventral (or vice versa), visibly inspect it for any signs of distress or changes in vitality.

Once the imaging procedure is completed, return the mouse to its cage where it will wake quickly.

**Note:* *Many prefer to inject into an awake animal. It is acceptable to sedate the mouse before injection, but doing so may slightly extend the kinetics (peak luciferase expression time).*

*For research use only. Not for use in diagnostic procedures*

**PerkinElmer, Inc.**
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
**www.perkinelmer.com**

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