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D-Luciferin *In Vivo* Protocol

D-Luciferin *in vivo* Protocol

Procedure for use with Gold Biotechnology D-Luciferin;
Catalog #: LUCK (Luciferin, Potassium Salt) and LUCNA (Luciferin, Sodium Salt)

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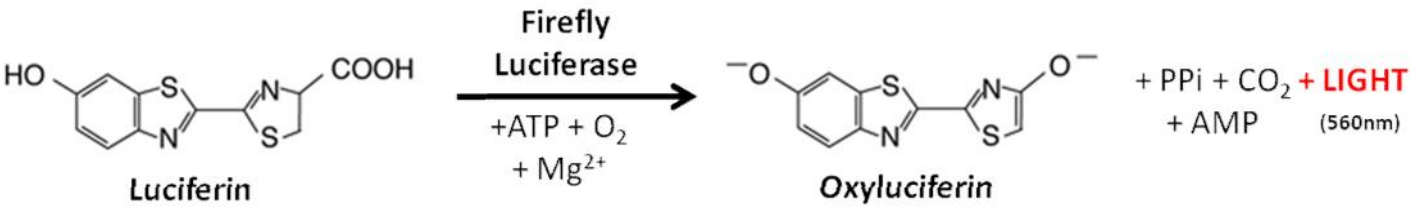
Editor - C. Menne (6/26/2014)

Gold Biotechnology
D-Luciferin *in vivo* Protocol

Introduction

Luciferin is a common bioluminescent reporter used for *in vivo* imaging of the expression of luciferase. This water soluble substrate for the Firefly luciferase enzyme utilizes ATP and Mg^{2+} as co-factors to emit a characteristic yellow-green emission in the presence of oxygen, which shifts to red light *in vivo* at 37°C. Through the utilization of ATP, the reaction can be further used to indicate the presence of energy or life in order to function as a life-death stain.

D-Luciferin is a common reagent used throughout the Biotechnology field and specifically for *in vivo* imaging. Luciferase labeled tumor cells, stem cells, or infectious diseases are often inoculated into research animals such as rats or mice for investigation. The injection of D-Luciferin allows for the real-time, non-invasive monitoring of disease progression and/or drug efficacy in these model systems through Bioluminescence Imaging (BLI).



Product Specifications

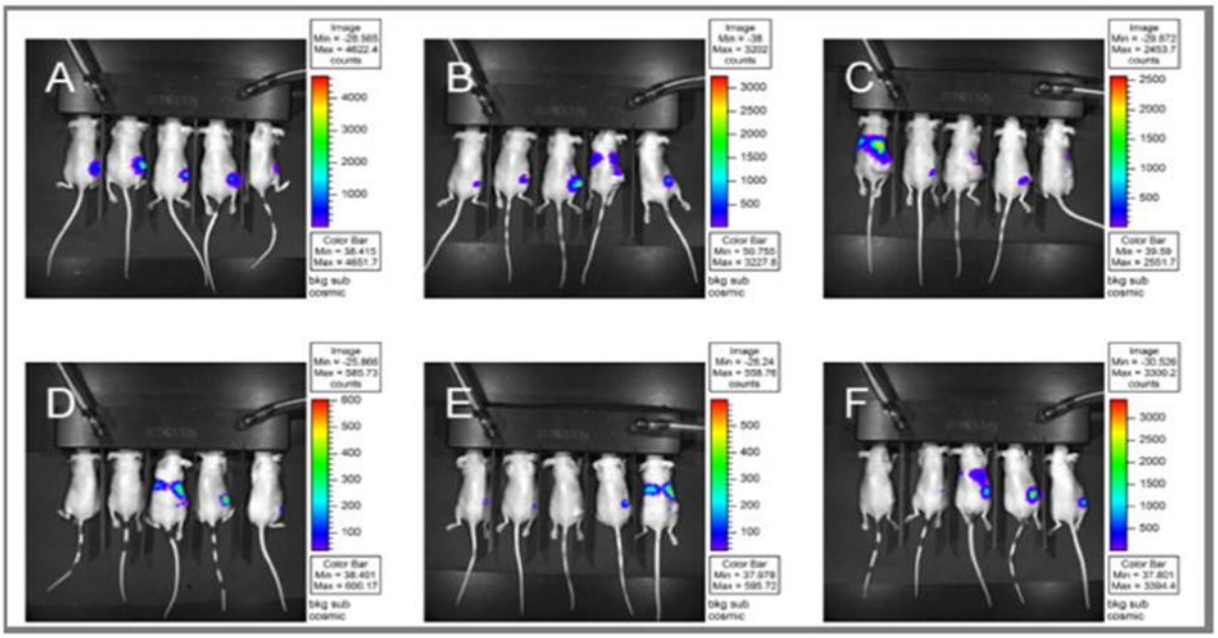
D-Luciferin, Potassium Salt
4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid potassium salt
$KC_{11}H_7N_2O_3S_2$
MW: 318.42 g/mol

D-Luciferin, Sodium Salt
4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid sodium salt
$NaC_{11}H_7N_2O_3S_2 \cdot H_2O$
MW: 320.32 g/mol

Storage/Handling: Store at -20°C. Protect from light.

Materials

- D-Luciferin salt
  - Luciferin, Potassium Salt (GoldBio Catalog # LUCK)
  - Luciferin, Sodium Salt (GoldBio Catalog # LUCNA)
- DPBS (Dulbecco's Phosphate-Buffered Saline), without Ca^{2+} or Mg^{2+}
- Syringe Filter, 0.2 µm



Bioluminescent images of mice injected with Ad.CMV.Luc + SELP815K at 4% (A–C) and free virus (Ad.CMV.Luc, D–F). Images are at days 4, 10, and 21 (left to right) by injection with 200 µl of 15 mg/ml luciferin intraperitoneally.
Gustafson, Joshua, *et al.* "Silk-elastinlike recombinant polymers for gene therapy of head and neck cancer: From molecular definition to controlled gene expression." *Journal of Controlled Release* 140.3 (2009): 256-261.

Luciferin Preparation

1. Thaw D-Luciferin (either Potassium or Sodium Salt) at room temperature and dissolve in DPBS (no calcium or magnesium) to a final concentration of 15 mg/mL.

2. Pre-wet a 0.22 µm filter by drawing through 5-10 mL of sterile H_2O and discard water.

3. Sterilize the Luciferin solution through the prepared 0.22 µm syringe filter.

4. Inoculate lab animal according to one of the **various methods detailed below**. (Luciferin is typically administered either intraperitoneally or intravenously.)

Example: Inject with 10 µL of Luciferin stock solution per gram of body weight (normally ~200 µL for a 20 g mouse for a standard 150 mg/kg injection).

5. Wait 10-20 minutes before imaging for maximum luciferase signal plateau.

6. A kinetic study of luciferase should be performed for each animal model to determine the peak signal time and plateau phase.

Determining the Kinetic Curve

The kinetics of tissue biodistribution may be different for each animal model and experimental design visualized. We recommend creating a kinetic curve for each system prior to conducting the experiment in order to determine the peak signal time for imaging the animal model after Luciferin injection.

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

1. Inject Luciferin via one of the proposed methods listed below (Intraperitoneal, Intravenous, or Subcutaneous). If you need to sedate the animals before injection, be aware that it may slightly extend the kinetics (peak luciferase expression time). The biodistribution of Luciferin may also be different depending on the route of administration.

2. **For IP or SQ Injection:**
    a. **If you are able to inject into animals which are still awake**, wait three minutes, then sedate by your method of choice (gas or injectable anesthesia). See Appendix for anesthesia/analgesia drugs and dosages in rats and mice.
    b. Place sedated animals in imaging chamber and take the first image approximately five minutes post Luciferin injection.

3. **For IV Injection:**
    a. Immediately sedate the animal (if not already sedated) by your method of choice (gas or injectable anesthesia). See Appendix for anesthesia/analgesia drugs and dosages in rats and mice.
    b. Place sedated animals in imaging chamber and take the first image within first two minutes post Luciferin injection.

4. Continue to take images every 5-10 minutes up to about 40-60 minutes for IP or SQ injected models (for IV injected models: image every 1-5 minutes up to 20-30 minutes) to generate a kinetic curve for luciferase expression in your model. (**Most injectable**

anesthesia will last 20-30 minutes and might require an additional dose for a full kinetic curve study!)

5. Choose the best time point to image your model from your kinetic curve. Many models reach their peak signal time approximately 10-20 minutes post IP or SQ Luciferin injection and 2-5 minutes post IV Luciferin injection.

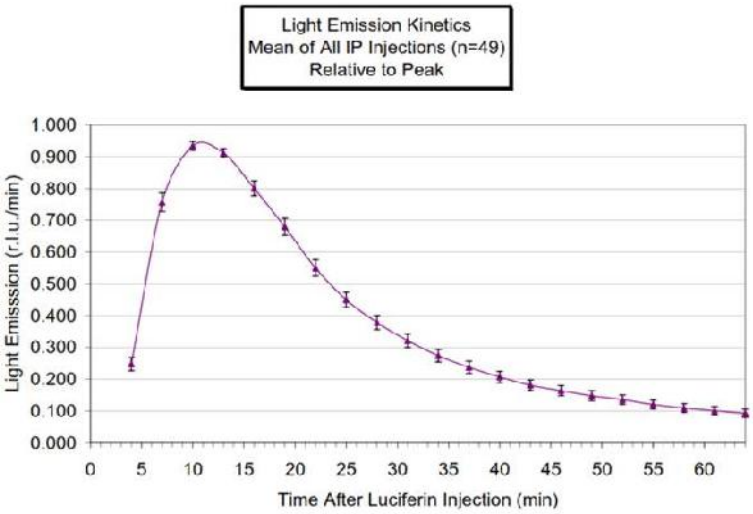


Figure 3.8 – i.p. luciferin injection
Integrated light relative to the peak of the kinetic curves

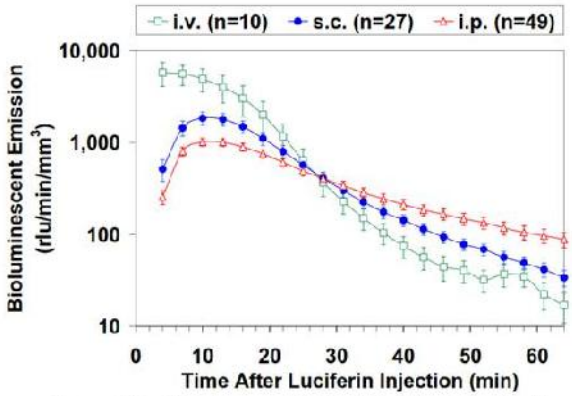
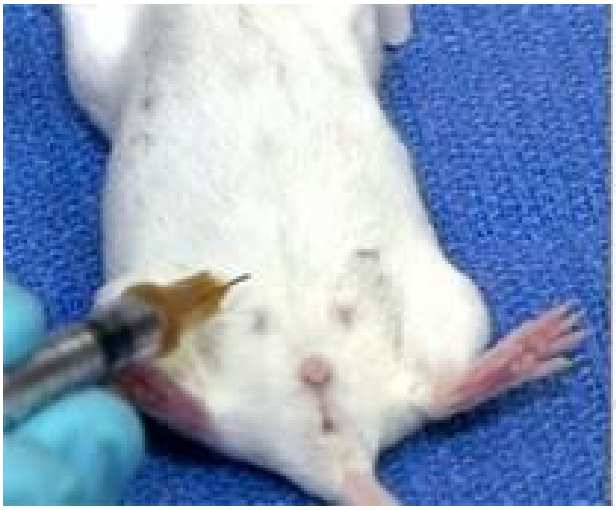


Figure 3.43 – Bioluminescent Light Emission Kinetic Profiles for Various Luciferin Injection Routes (Vol 2) - BLI emission decays exponentially for each injection route, but at very different rates. After 43 minutes, the BLI emission following i.v. injection approaches the level of background noise which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

Intraperitoneal (IP) Injection



Mouse

1. First locate the point of entry for the needle.
2. Draw an imaginary line across the abdomen just above the knees (see image above).
3. The needle should be inserted along this line on the animal's right side and close to the midline. (The point of entry is cranial to and slightly medial of the last nipple in a female mouse.)
4. Inserting the needle on the mouse's right side avoids the cecum, which is a large fluid-filled organ on the left side of the abdomen. The small intestines (on the right side) are less likely to be punctured by the needle.
5. Inserting the needle too far caudally or laterally from the insertion point shown above would risk making an injection into the rear leg which would injure the muscle tissue.
6. To perform an IP injection, the mouse must be well restrained so that it cannot move during the procedure. This avoids traumatizing the organs once the needle has entered the abdomen.
7. Restrain the mouse and tilt so that the head is facing downward and its abdomen is exposed.
8. Thoroughly disinfect the injection site with 70% ethanol.
9. We recommend using a 25-27 gauge needle for IP injections. Insert the needle into the abdomen at about a 30-degree angle.

10. The shaft of the needle should enter to a depth of about a half centimeter (4-5 mm).
11. Aspirate slightly to be sure that the needle has not penetrated a blood vessel, the intestines, or the urinary bladder.
    a. Greenish brown aspirate indicates needle penetration into intestines.
    b. Yellow aspirate indicates needle penetration into the bladder
12. If any fluid is aspirated, your solution is contaminated and **must** be discarded and the procedure repeated with a new syringe and needle.
13. If no fluid is aspirated, you may inject.
14. Withdraw the needle and make record the injection time to determine peak signal time.

Rat

Follow mouse IP procedure. We recommend a 25 gauge needle for IP injections in the rat.

Tips

- Make sure that the bevel of the needle is pointed up.
- Tilt the animal head down in order to allow the intestinal content to move downward, leaving an empty cavity in the lower abdominal quadrant.
- To ensure accurate injection depth and prevent deep injections, we recommend placing a piece of catheter tubing over the needle leaving the appropriate 4-5 mm exposed for proper depth.
- Ensure proper volume of injection. We recommend no more than 2 ml in mice and no more than 5 ml in rats. (See Appendix for injection volumes by site and animal.)
- When injecting rats, covering the rat's head with a cloth will prevent stress on both the mouse and researcher.

Intravenous Injection

Mouse

Tail Vein Injection

Restrain the mouse with physical or chemical restraint (See Appendix for various anesthesia/analgesia drugs and dosage for mice and rats). Rotate the tail slightly to visualize vein. Disinfect injection site with 70% ethanol and insert the needle (27-30 gauge) into the vein at a slight angle. **You will not be able to aspirate**, instead inject slowly and watch for clearing of the lumen. Incorrect positioning will result in a slight bulge in the tail. If this occurs, remove needle and repeat process proximal to previous site. Upon completion, remove needle and apply pressure to injection site. Record the injection time to determine peak signal time.

Tail Vein Injection - Further details

Before making an injection in a tail vein, it is helpful to induce peripheral vasodilatation and make the vein more prominent for cannulation. You can raise the mouse's body temperature with an incandescent lamp, soak the tail in warm water, or administer vasodilatating agents such as xylazine or acepromazine. You can also use a light source to transilluminate the tail which will make the vein easy to see and access.

A safe water temperature is 110°F or 43°C, which will gently warm the mouse. Although warming an anesthetized mouse is beneficial, be careful not to cause burns or overheat the animal.

The tail veins are located on each side of the tail, superficially just under the skin. When the needle is correctly placed in the vein, you may or may not see a flash of blood in the needle hub. You will get a sense that the needle has entered the vein because **there will be less resistance to the advance of the needle** inside the vein's lumen than through the subcutaneous tissues. Likewise, upon injection, **the fluid will flow easily into the vein**, and the vein will become clear (changing from dark to light) as the fluid temporarily replaces the blood (as in the image above).

Rat

Tail or Saphenous Vein Injection

Use tail injection procedure same as in the mouse.

1.

The animal is placed in a restraining device and the tail is warmed in a water bath. Take care not to overheat the animal.

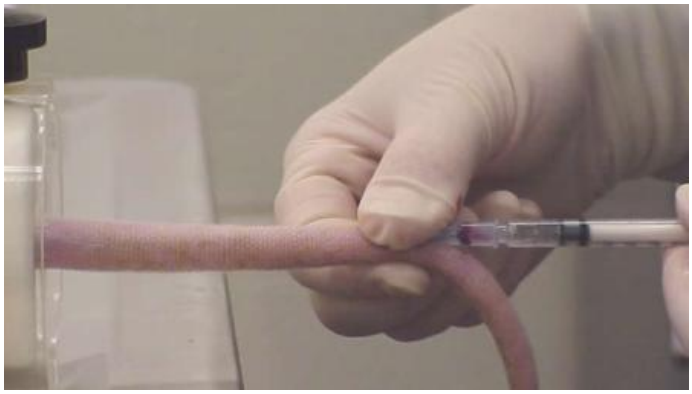
2.

Pull the tail snugly and rotate it to position the vein on top. Insert the needle about 1 cm from your fingers.

3.

After penetrating the skin surface, reposition the syringe parallel with the tail. Inject slowly.

4.



Hold the thumb over the injection site prior to withdrawing the needle to prevent bleeding.

For the **saphenous vein**:

1. Restrain the rat with the use of anesthesia (See Appendix for various anesthesia/analgesia drugs and dosage for mice and rats).
2. Extend the hind leg and shave hair to expose lateral saphenous vein.
3. Disinfect the injection site and apply tourniquet-like pressure to the upper portion of the leg.
4. Insert needle into the vein and aspirate.

5. Release tourniquet pressure and inject.
6. Upon completion remove needle and ensure proper hemostasis.
7. Record the injection time to determine peak signal time.

We recommended using a 22-25 gauge needle size for IV injections in the rat.

Tips

- **Be sure there are no air pockets or bubbles in the solution to be injected, as this can kill small animals.**
- Restrain animal firmly but gently.
- Do not aspirate.
- Inject **slowly**.
- Warming the tail in water will make the vein easier to see.
- The fluid should flow easily into the vein. If it does not, reset the needle and try again.
- After injection, apply pressure with thumb on the injection site briefly to help prevent bleeding.

Subcutaneous Injection

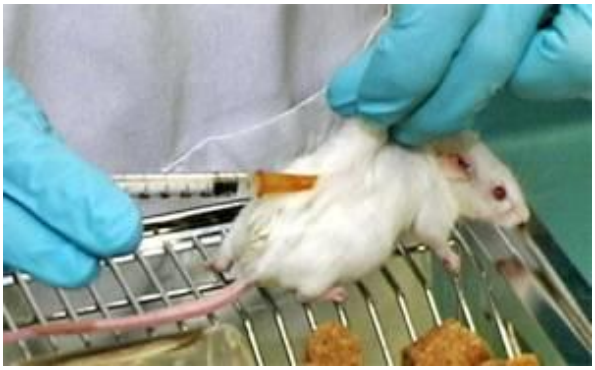
Mouse

Subcutaneous injections are used often to inject anesthetics or to administer fluids for hydration during anesthesia recovery. The subcutaneous route of injection is often abbreviated as SC or SQ. The amount of fluid injected should be limited to volumes that will not overly stretch the skin (which would be uncomfortable) or that will not over-hydrate the animal unnecessarily. Typical volumes injected subcutaneously are in the range of 1 ml or less.

- The most common injection site is the loose skin around the neck and shoulder area.

The mouse should be restrained in the normal manner. With your fingers, lift the skin to make a "tent". Disinfect the injection site with 70% ethanol. We recommend using a 22 gauge needle for subcutaneous injections.

Procedure



1. Lift the skin over the back to form a tent. (If mouse is awake, place the mouse on the wire lid so it can hang on with its front paws during the injection.)

2. Scruff the skin over the back and tent it up. Your hand is both restraining the mouse and presenting the area to be injected.

3. Insert the needle at the tent base, being careful to avoid directing the needle at your fingers. Your fingers should be at top of the tent, safely above the point of the needle's entry. **Hold the needle parallel** to the animal's body to also avoid puncturing underlying structures.

4. Aspirate to ensure that the needle has not entered a blood vessel. **Proper placement should yield no aspirate**.

5. Inject the full volume at a moderate rate.

6. Withdraw the needle and then press the skin to seal the needle's exit hole in the skin and to prevent the fluid from leaking out.

7. Check the animal for any bleeding. Because the fluid has been deposited in the subcutaneous space, you can see and feel the bubble of fluid, called a bleb.

8. Record the injection time to determine peak signal time.

Mice will generally not object to a subcutaneous injection when they are allowed to grasp the wire lid.

Rat


Follow mouse subcutaneous injection method, but hold the rat against the table instead of placing on wire cage. We recommend using a 23-25 gauge needle and injecting no more than 5 ml of fluid.

- Most common injection site is the loose skin around neck and shoulder area.

Tips

- Make sure that the bevel of the needle is pointed up when injecting.
- When injecting rats, covering the rat's head with a cloth will prevent stress on both the mouse and researcher.
- Gently massaging the injection site will help to dissipate the bleb.

Appendix

Table 1: Specific Recommended Injection Sites, Volumes and Needle Sizes for Mice

ROUTE	SITE	MAXIMUM VOLUME	MAXIMUM NEEDLE SIZE
SQ	Scruff	1 mL	22 gauge
IM	Caudal Thigh	0.05 mL	25 gauge
IP	Lower Ventral Quadrants	2 mL	25 gauge
ID	Lateral Abdomen/Thorax	0.05 mL	27 gauge
IV	Lateral Tail Vein	0.5 mL	27 gauge

Table 2: Suggested Sites, Needle Sizes and Injection Volumes for Various Routes and Model Animals (*Credit*: *Harkness and Wagner's "Biology and Medicine of Rabbits & Rodents."*)

Route		Gerbil	Hamster	Mouse	Rat
IM	**Site**	Quadriceps	Quadriceps, gluteals	Quadriceps	Quadriceps, gluteals, triceps
	Volume	≤0.03 mL	≤0.15 mL	≤0.03 mL	≤0.25 mL
	Size	<23 gauge	<23 gauge	<23 gauge	<22 gauge
IP	**Site**	Lower right quadrant of abdomen	Lower right quadrant of abdomen	Lower right quadrant of abdomen	Lower right quadrant of abdomen
	Volume	≤2 mL	≤3 mL	≤2 mL	≤5 mL
	Size	<25 gauge	<21 gauge	<25 gauge	<22 gauge
IV	**Site**	Lateral tail veins	Not Recommended	Lateral tail veins	Lateral tail or saphenous veins
	Volume	≤0.5 mL		≤0.5 mL	≤2 mL slowly
	Size	<23-gauge		<27 gauge	<22 gauge
Intra-gastric	**Site**	Stomach	Stomach	Stomach	Stomach
	Volume	N/A	N/A	5-10 mL/kg	5-10 mL/kg
	Size	18-22 gauge 2-3 cm long Bulbed feeding needle	18-22 gauge 4-4.5 cm long Bulbed feeding needle	18-22 gauge 2-3 cm long Bulbed feeding needle	15-18 gauge 6-8 cm long Bulbed feeding needle or 8 French flexible catheter
SQ	**Site**	Neck, back	Neck, back	Neck, back	Neck, back, abdomen
	Volume	≤1 mL	≤3 mL	≤1 mL	≤5 mL
	Size	<22 gauge	<21 gauge	<22 gauge	<21 gauge

Table 3: Various Drugs Used for Anesthesia/Analgesia of Rats and Mice.
(IP = intraperitoneal; SC = subcutaneous; PO = per os (by mouth); IV = intravenous)

Drug	Rat Dose (mg/kg)	Mouse Dose (mg/kg)	Comments
Tranquilizers/Sedatives			
Acepromazine	2.5 IP	2.0-5.0 IP; SC	Light sedation
Diazepam	2.5-5.0 IP	5 IP	Light sedation
Midazolam	5.0 IP	5.0 IP	Light sedation
Xylazine	1.0 – 5.0 IP	5.0 – 10.0 IP	Light to heavy sedation; some analgesia
Anticholinergics			
Atropine	0.05 IP; SC	0.04 SC	Reduces secretions; protects heart from vagal inhibition
Glycopyrrolate	0.5 IM		Reduces secretions; protects heart from vagal inhibition
Analgesics			
Buprenorphine	0.01-0.05 SC q8-12h	0.05-0.10 SC q8-12h	For moderate to severe pain
Butorphanol	1.0-2.0 SC q2-4h	1.0-2.0 SC q4h	For moderate to severe pain
Ketoprophen	5.0 SC or PO	?	For moderate pain
Meloxicam	1.0 SC or PO	?	For moderate pain
Injectable Anesthetics			
Ketamine + xylazine	75-100 + 10 IP	80-100 + 10 IP	20-30 minutes surgical anesthesia
Ketamine + medetomidine	75 + 0.5 IP	75 + 1.0 IP	20-30 minutes surgical anesthesia
Ketamine + acepromazine	75 + 2.5 IP	100 + 5 IP	20-30 minutes light anesthesia
Ketamine + diazepam	75 + 5 IP	100 + 5 IP	20-30 minutes light anesthesia
Ketamine + midazolam	75 + 5 IP	100 + 5 IP	20-30 minutes light anesthesia
Pentobarbitol sodium	45-50 IP	50-90 IP	anesthesia
Propofol	10 IV		5 minutes surgical anesthesia

References

All injection methods provided by AALAS Learning Library

Pictures

1. Gustafson J, *et al*. (2009). *Journal of Controlled Release*, *140*(3), 256-261.
2. R.A. Bollinger. Dissertation: 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.
3. Boston University, Animal Care Research Compliance.
4. Boston University, Animal Care Research Compliance.
5. LSSU - School of Biological Sciences – Institutional Animal Care and Use Committee.
6. Boston University, Animal Care Research Compliance.
7. LSSU - School of Biological Sciences – Institutional Animal Care and Use Committee.

Tables

1. US National Institute of Health
2. Adapted from Harkness and Wagner's "*Biology and Medicine of Rabbits & Rodents.*"
3. Saskatchewan Association of Veterinary Technologists (SAVT)