
 <b>University of Zurich</b> <sup>UZH</sup> Institute of Laboratory Animal Sciences	<b>Standard Operating Procedure</b>  <b>SOP</b>	<b>Page 1 of 3</b>
<b>Date:</b> 16.09.2022	<b>Bioluminescent in vivo imaging</b>	<b>LTK-RES-6-B-EN</b> <b>Version: B</b>
<b>This SOP replaces:</b> Date: 12.07.2019 Version: A		
<b>Reason for Change:</b> Change of title		
<b>Related SOPs:</b> SOP-LTK-RES-3 Stereotactic Injection SOP-LTK-RES-4 Implantation of osmotic minipumps SOP-LTK-TRT-13 Isoflurane anesthesia		
<b>Indication of Use:</b> Bioluminescent or fluorescent markers within a mouse		
<b>Aim of SOP:</b> This protocol describes how to perform non-invasive fluorescent or bioluminescent in vivo imaging using a Perkin Elmer IVIS imaging device. It is also applicable for imaging devices from other companies.		
<b>Distribution:</b> 1. Server 2. Animal facility 3. Group vom Berg		
<b>Attachments:</b>		
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**Responsible Persons:**

- 1) The researcher mentioned on the respective scoring sheet
- 2) Any person with Module 1 and registered on animal permit

**Method:** Non-invasive detection of photons emitted from a mildly anesthetized live animal

**Principle of Method:** Charged-coupled device (CCD) cameras have highly sensitive sensors that can pick up photons from deep within living specimens.

**Material to be used:**

Vit A eye ointment / humigel

*Bioluminescent imaging:*

- D-Luciferin, Firefly, potassium salt, 1.0 g/vial,
- DPBS, w/o  $Mg^{2+}$  and  $Ca^{2+}$

Prepare a fresh stock solution of Luciferin at 15 mg/mL in DPBS. Filter sterilize through a 0.2 um filter. Aliquot solution in 1.5 ml tubes of black or brown color to avoid bleaching.

*Fluorescent imaging:*

FMT tracers (e.g. from Perkin Elmer, follow technical data-sheet for reconstitution)  
transgenic mice or modified cells

**Storage of Material:** Store lyophilized or reconstituted luciferin at -20°C (refreeze for maximal 3x, tick-mark lid for every thawing). For FMT tracers follow the individual technical data sheets

**Machine:** Perkin Elmer *IVIS* or *FMT* Systems or similar


**Material:**

Luciferin or FMT tracer, transgenic reporter cell line or transgenic animal

**Safety:**

1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Follow the rules of the animal house

File: SOP-LTK-RES-6-B-EN In vivo imaging

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**Method Description:**

1. Open software (*Living Image* by Perkin Elmer), log in and start system initialization) and heat the platform to 37°C. While initiation sequence is running, weigh mice and calculate amount of D-luciferin needed.
2. Inject up to 200 µl of imaging solution (150 mg/kg body weight D-luciferin) intraperitoneally (i.p. D-luciferin) or up to 100 µl intravenously (i.v., some FMT tracers, check according to datasheet) **SOP LTK-TRT-10 Intraperitoneal injection** or **SOP LTK-TRT-7 Intravenous injection**, note down the time of injection/start a timer
3. Anesthetize mice using 3.5% isoflurane in an inhalation chamber (**SOP-LTK-TRT-13 Isoflurane anesthesia**)
4. Remove the mice from the inhalation chamber and shave the head gently using a small animal hair-trimmer, in case animal is about to wake up, put it back into the induction chamber, repeat until head is shaved
5. Cover eyes with eye ointment
6. Transfer the animals into the imaging device and apply isoflurane and oxygen through the nose nozzle, reduce isoflurane down to 1.5% (if possible, in some cases 2% is necessary for maintenance of anesthesia) for acquisition, goal is to have as little movement artifacts as possible
7. Wait 10 min for the tracer/luciferin to evenly distribute in the animal
8. Image mice for up to 30 minutes.
9. Gently remove mice from the imaging device and place into a clean cage.
10. Monitor the mouse until recovery (**SOP-LTK-TRT-13 Isoflurane anesthesia**)

**Documentation:**

Server, appropriate project folder, imaging alone is severity 1 and has to be recorded in iRATS

**Problem management:**

Report any adverse event to your supervisor