 University of Zurich <small>UZH</small> Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 1 of 4
Date: 13.07.2016	In vivo imaging	LTK-RES-6-A-EN Version: A
This SOP replaces: Date: None Version: None		
Reason for Change: None		
Related SOPs: SOP-LTK-RES-3- EN Stereotactic Injection SOP-LTK-RES-4- EN Implantation of osmotic minipumps SOP-LTK-TRT-13- EN Isoflurane anesthesia		
Indication of Use: Bioluminescent or fluorescent markers within a mouse		
Aim of SOP: 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.		
Distribution: 1. Server 2. Animal facility 3. Group vom Berg		
Attachments:		
Generated at: 30.08.2015	Checked and approved at: 15.10.15	
by: Johannes vom Berg	by: Thorsten Buch	

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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 (Caliper Life Sciences Part Number #119222),

- DPBS, w/o Mg²⁺ and Ca²⁺

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


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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. Anesthetize mice using 3.5% isoflurane in an inhalation chamber (**SOP-LTK-TRT-13-A-EN Isoflurane anesthesia**)
3. Remove the mice from the inhalation chamber and shave the head and back 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
4. Inject up to 200 µl of imaging solution (150 mg/kg body weight D-luciferin) intraperitoneally (i.p. D-luciferin) or intravenously (i.v., some FMT tracers, check according to datasheet) **SOP LTK-TRT-10-A-EN Intraperitoneal injection** or **SOP LTK-TRT-7-A-EN Intravenous injection**, note down the time of injection/start a timer
5. Cover eyes with eye ointment
6. Transfer the animal 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-A-EN Isoflurane anesthesia**)

Documentation:

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

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Problem management: Report any adverse event to your supervisor
