This SOP replaces:	Date: 15.10.2015 Version: A
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

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

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-C-EN Intraperitoneal injection** or **SOP LTK-TRT-7-B-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

Problem management:
Report any adverse event to your supervisor