

Ex vivo tissue Bioluminescence Imaging (BLI)

Materials needed:

- IVISbrite™ D-Luciferin potassium salt bioluminescent substrate (Firefly), (Revvity catalog # 122799).
- 24 well plates
- ATP 1 mM
- DPBS, without Mg²⁺ and Ca²⁺
- Syringe filter, 0.2 µm

Procedure:

1. Prepare the following two concentrations of IVISbrite D-luciferin in DPBS and filter sterilize with a 0.2 µm filter:
 - 15 mg/ml for injection *in vivo* prior to euthanasia
 - 300 µg/ml for imaging *ex vivo* tissues
2. Just prior to euthanasia, mice are injected at 150 mg/kg with the 15 mg/ml stock of IVISbrite D-luciferin. (Depending on the luciferin kinetics (plateau phase) allow 5 -15 min of luciferin absorption).
3. Immediately after necropsy, tissues of interest are placed individually into wells of a 24-well plate.
4. If you image the *ex vivo* tissues within 45 minutes post IVISbrite D-luciferin injection, it is usually not necessary to add IVISbrite D-luciferin and ATP. However, if necessary, cover the tissues in a solution with IVISbrite D-luciferin (300 µg/ml) and 1mM ATP*.

5. Tissues are imaged initially at 1 minute, 10 bin, FOV 15. Image times and binning can then be adjusted accordingly.
6. Tissues can then be fixed in 10% formalin and H&E stained for histology.

Notes:

- False positives can be seen when there is carry over of signal from bright tissues to negative tissues. This carry over may occur when bright tissues happen to be placed in wells adjacent to negative tissues. In this case, the bright tissue can be removed from the plate and the remaining tissues re-imaged.
- It is helpful to separate the lobes of the lungs and liver and place them in separate wells as dim signals can be attenuated.
- It is important to try to image the tissues as soon as possible after they are removed from the animal to minimize tissue degradation. ATP decreases rapidly in dead tissue and becomes the limiting co-factor in the luciferase reaction.

*Prepare a 0.55 mg/ml (1mM) solution of ATP, such as Sigma-Aldrich Product Number A2383