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# Bioluminescence Imaging

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## Abstract

This protocol describes a bioluminescence stepwise imaging protocol in mice with AAV-luciferase expression in the vagal afferents using the lvis Lumina series III imaging system.

## Materials

- D-luciferin potassium salt (Xenogen)
- Isoflurane, USP (Covetrus)



## Preparation and In vivo imaging

- 1 Induce anesthesia with isoflurane 2-3%.
- 2 Shave the neck, thoracic and abdominal area.
- Prepare D-luciferin potassium salt (Xenogen) stock prepared in phosphate buffered saline (PBS) at 15mg/mL.
- 4 Inject D-luciferin solution (150mg/kg) intraperitoneal.
- 5 Image mice in supine position at 5-, 10- and 15-minutes post-luciferin injection.

## Software protocol and analysis

- 6 Living imaging software (Ivis imaging) was configured to luminescent imaging mode with an exposure time of 00:00:00 and 5-minute delay.
- Post-acquisition, the total flux of photons/radiance per second (s) in each pixel in the ROI area, in a square centimeter (cm<sup>2</sup>) of the tissue. This data is then multiplied by one steradian (sr), which result in average radiance displayed as (photons/s/cm<sup>2</sup>/sr).