TECHNICAL BRIEF

Bioware and Bioware Ultra oncology cell lines

Cell preparation and imaging protocol

Intravenous model

Materials

- Bioluminescent oncology cells
- Mice
- Artagain black paper (Strathmore, Catalog #445-109)
- Luciferin (for *in vivo* and *ex vivo* imaging)
- Syringe (1 mL) and needles (25 x 5/8" gauge and 26 x 1/2" gauge)
- Heating lamp and mouse restrainer (for cell injection)
- Anesthesia (isoflurane or ketamine/xylazine)
- Forceps and scissors (for necropsy)
- 24 well plates (for *ex vivo* imaging)

Protocols

Preparation of tumor cells:

Cells are trypsinized from T175 flasks, and resuspended in DPBS at a recommended concentration, typically 1 x 10^6 tumor cells/100 uL DPBS.

Injections:

1. On Day 0, mice are injected with firefly luciferin (150 mg/kg) by intraperitoneal injection using a 25 x 5/8” gauge needle. (See “I.P. Injection of Luciferin”)
2. Mice are placed under a heating lamp till tail vein dilation (4-5 minutes).
3. Mice are placed into a restrainer and tumor cells (typically 5x10^6 cells in 100 uL DPBS per mouse) are SLOWLY injected (2-3 minutes) via i.v. through the tail vein using a 26 x 1/2” gauge needle.
4. Mice are anesthetized by gas anesthesia (3% isoflurane) or by intramuscular injection of ketamine/xylazine using a 25 x 5/8” gauge needle. (See “Anesthesia Protocol”)
5. Mice are placed onto a black paper in the IVIS® imaging box and imaged dorsally and ventrally.

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Imaging:

Animals are imaged after injection with D-luciferin over a period of time ranging from 1 month to 3 months (see Determining Kinetic Curve for Luciferin protocol). At the end of experiment, animals are euthanized and selected tissues are analyzed by *ex vivo* imaging and then processed for subsequent histology. (See "*Ex Vivo* Imaging Protocol")

Important notes

Order and timing of injections and imaging:

a. Luciferin is given first by i.p. injection (refer to luciferin injection protocols).
b. On day 0, animals are placed under a heat lamp for 4-5 minutes, and cells are injected i.v. via tail vein slowly (another 2-3 minutes). Animals are anesthetized (another 2-3 minutes), then imaged.
c. On subsequent days, luciferin is re-injecting and after 7-8 minutes, animals are anesthetized (another 2-3 minutes), then imaged. Thus, imaging takes place at about 10 minutes after luciferin injection.

** A luciferin kinetic study should be performed for each model to determine peak signal time.

Imaging times:

a. For the IVIS® system, images are typically 2 minutes, 10 bin at level B on Day 0. As metastases develop and signals get brighter, the image time can be reduced to a few seconds.
b. For image acquisition, we advise using "counts." This enables the user to adjust camera settings to optimize the signal level. The signal should be well above the background noise (~100 counts) and below the saturation value (65535 counts).
c. For quantification of signals using ROI measurements or comparison of several images for presentation purposes, we recommend using "photons." In this mode, the measurements or image displays automatically take into account settings of such as integration time, binning, f/stop, and field-of-view.

Bioluminescent signals and metastatic sites:

Day 0: If the tail vein injection is "good", one will see a signal in the lungs on Day 0. Unsuccessful injections will be obvious on Day 0 since the bioluminescent signal will be limited to the tail region of the mouse.

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