TECHNICAL BRIEF

Bioware and Bioware Ultra oncology cell lines

Cell preparation and imaging protocol

Subcutaneous model

Materials

- Bioluminescent oncology cells
- Mice
- Artagain black paper (Strathmore, Catalog #445-109)
- Luciferin (for *in vivo* and *ex vivo* imaging)
- Syringe (1ml) and needles (25 x 5/8" gauge)
- Forceps and Scissors (for cell injection and necropsy)
- Anesthesia (isoflurane or ketamine/xylazine)
- Aluminum foil (for shielding primary tumor, see spontaneous metastasis section)
- 24 well plates (for *ex vivo* imaging)

Protocols

Preparation of tumor cells:

Cells are trypsinized from T175 flasks, and resuspended in DPBS, typically at a concentration of ~1x10^6 cells in 100 uL.

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. After 7-8 minutes, 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)
3. Tumor cells (typically 1 x 10^6 cells in 100 uL DPBS) are injected subcutaneously into the mouse.
4. Mice are placed onto black paper in the IVIS™ imaging box and imaged dorsally and ventrally.

PerkinElmer®
For the Better

Imaging:

For detection of primary tumor:

a. Mice are imaged from dorsal view.
b. Signal at the injection site will be detectable at day 0. Measurable tumors (50-100 mm^3) can be calipered after ~2-3 weeks.

For detection of spontaneous metastases:

a. The primary tumor should be shielded (to detect metastatic signals) and mice are imaged on the ventral side. Shielding can be done by wrapping aluminum foil around the lower abdominal area of the mouse. Black paper is also placed on top of the foil to eliminate reflected light.
b. Initial metastatic signals from thoracic area begin to appear after a few to several weeks. Other metastases can be detected by *ex vivo* imaging.
c. 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 kinetic study should be performed for each model to determine peak signal time.

a. Luciferin is given first by i.p. injection (approximately 200 ul for a 20 g mouse).
b. On day 0, after 7-8 minutes, animals are anesthetized (another 1-2 minutes). Cells are injected (another 2-3 minutes) and animals are then imaged.
c. On subsequent days, D-luciferin is re-injected and after 10 minutes, animals are anesthetized (another 1-2 minutes), and then imaged. Thus, imaging takes place at ~10-15 minutes after luciferin injection.

Imaging times:

a. For the IVIS™ system, images are typically 1 minute, 10bin at level B on day 0. As tumors are growing and signals get brighter, the image time can be reduced to 10 seconds. For detection of metastases, while the primary tumors are shielded, images are usually taken for 3-5 minutes, 10bin, at level B or A. Image time can also be reduced depending on the intensity of metastatic signals.
b. For image acquisition, we advise using " counts." This enables the user to adjust camera settings to optimize the signal level within the range allowed by the digitizer. 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 purpose, we recommend using "photons." In this mode, the measurements or image displays automatically take into account the various settings of integration time, binning, f/stop, and field-of- view.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs