STANDARD OPERATING PROCEDURE

Cat K™ 680 FAST (NEV11000)

IN VIVO IMAGING PROTOCOL

PREPARING MICE FOR IMAGING

- Animal hair is highly effective at blocking, absorbing, and scattering light during optical imaging. Even light within the NIR spectrum, which typically shows minimal scattering and absorbance in tissue, is significantly absorbed and scattered by hair.
- Always remove the fur on and around the areas of the animal that are to be imaged. For quantitative accuracy, continue this practice throughout the study. Remove all hair from mice when performing 3D imaging on IVIS or FMT.
- Nude mice, or immunocompetent hairless SKH1-E mice, do not require depilation; however conventional strains of haired mice, like BALB/c or C57BL/6, require depilation.
- 2 weeks before the imaging study switch to low fluorescence mouse chow. Regular mouse chow contains chlorophyll that auto-fluoresces around 700 nm and can interfere with signal collected from this agent.

PREPARING CAT K 680 FAST

- Each vial contains 24 nmol of Cat K 680 FAST in a dry solid form.
- Add 1.2 mL of 1 x PBS to the vial and gently shake or vortex to ascertain the agent is in solution.
- This material provides sufficient reagent for imaging approximately 10 mice (weighing ~25 grams each) when using the recommended dose of 2 nmol/100 µL of Cat K 680 FAST per mouse.
- Once reconstituted with 1 x PBS, the solution is stable up to 14 days when stored at 2-8 ºC and protected from light.

ANIMAL INJECTION AND IMAGING

- Take a time-zero image of each subject before injection of the agent.
- With fluorescent models, a naïve control is fundamental and will allow for easier differentiation of signal from background, also facilitating advanced background subtraction features such as spectral unmixing on IVIS platform.

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- The imaging agent route of injection is an extremely important consideration when imaging fluorescence. In general, NIR imaging agents are designed for intravenous injection and are not optimized for injections by any other routes (e.g. intraperitoneal, intramuscular and subcutaneous).
- Inject 100 μl of Cat K 680 FAST intravenously into the mouse.
- The optimal imaging time point is **6-24 hours** post injection of the agent. It is recommended to do an initial time course imaging study to determine the optimal imaging time for the experimental model.
- Tissue half-life of Cat K 680 FAST is 36 hours. The optimal re-injection time is 3 days, which allows for the complete clearance of the agent from the mouse.

IVIS IMAGING

- If using the 2D or epifluorescence feature of IVIS, animal positioning is important. Always place the animal in a position where the source of light (fluorescent signal) is closest to the detector or, if unknown, image both sides of the animal.
- Filter Set selection
    - IVIS Spectrum/Spectrum CT: 675 ex/720 em
    - IVIS Lumina: 675 ex/Cy5.5 em (standard Lumina filter set) or 640 ex/700 em (optional 600 series filter) or 675 ex/720 em (optional 700 series filter)
- If multiple animals are imaged at the same time use guards between animals to prevent signal contamination from neighboring animals.
- For imaging studies where signal is at depth (e.g. lungs, heart etc.) use trans-illumination or 3D tomography (FLIT), available on IVIS Spectrum and IVIS Spectrum CT.
- For further information on trans-illumination or FLIT please refer to tech notes under the help tab of the Living Image (LI) software

FMT IMAGING

- Animal positioning within the animal imaging cassette is very important. Over the course of a study, always maintain consistency with animal positioning.
- Use the height adjustment knobs on the cassette to make sure that the animal is gently but securely compressed. The animal should be flat against both windows of the imaging cassette.
- When sliding the cassette into the docking station always orient the cassette such that the fluorescent signal is closest to the camera or, if unknown, image both sides of the animal.
- When setting up a scan with TrueQuant software be sure to draw the scan region sufficiently large so that tissue surrounding the anticipated area of fluorescence is captured. Ideally, aim for a total of 30 to 50 scan points with at least 2-3 scan points surrounding (on all sides) the anticipated area of fluorescence. If necessary you can change the spacing between adjacent scan points (the source density) using the Advanced Scan Settings.
- Laser channel selection:
    - The correct laser channel should be properly pre-selected based on the experimental design you had previously set up in the Experiment Tab.
    - For Cat K 680 FAST, verify that the ‘Ch 680’ laser channel is selected

Learn more at www.perkinelmer.com/invivoreagents or contact us at invivoreagents@perkinelmer.com

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