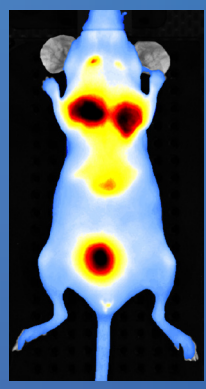
FLUORESCENCE IMAGING PANELS



Fluorescent Panel User Guide

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FLOURESCENCE MOLECULAR IMAGING

PerkinElmer's imaging probes are developed through an extensive R&D process and designed to incorporate drug-like biodistribution properties for optimal target delivery and performance. The table summarizes proper dosages, imaging time points, routes of metabolism, and probe clearance kinetics. Probes can be used either singly or in pairs by combining appropriate pairs of 680 nm and 750 nm probes. These probes can also be used for longitudinal studies by working within the parameters of the tissue pharmacokinetics and only reinjecting upon complete clearance.

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Protocol For Monoplex, Multiplex, and Longitudinal Imaging with *In Vivo* Probe Panels

STUDY PREPARATION

1. Two weeks before the imaging study, switch mice to low fluorescence chow. Regular mouse chow contains chlorophyll that auto fluoresces around 700 nm which can interfere with imaging.
2. On the study day, it is essential to prepare ahead of time for optimal results. Group and number the mice to be injected and imaged. Appropriate study design should include both positive control and negative control (i.e. un-diseased) mice injected with probe(s).
3. Mouse hair removal is essential for sensitive, high-quality fluorescence imaging. Either genetically hairless mice (SKH-1E) or normal, haired mice (BALB/c, C57BL/6, etc.) with depilation, must be used for optimal fluorescence tomographic imaging. This can be performed under injectable or inhaled anesthesia.
   - To minimize light scattering and absorption in fluorescence imaging, hair is removed from the appropriate body region of all mice; depilatory cream (Nair lotion, Church and Dwight Co., Inc., Princeton, NJ) is applied thickly on hair over the imaging region of each mouse, rinsed off thoroughly with warm water, and reapplied until all hair has been removed. [Rinsing must be done carefully and thoroughly to minimize any introduction of skin lesions that can cause imaging artifacts.]
   - Care should be taken to remove hair from an area larger than just the region of focus to assure that you can capture target and surrounding background fluorescence. For tomographic imaging, hair must be removed from front, sides, and back for the region of focus.
4. Establish the readiness of the imaging system by checking the anesthesia chamber and connections to the system. Activate the anesthesia, setting evaporator to the appropriate settings for you particular set-up.
5. Make sure you know ahead of time the proper positioning of the mouse you will use to facilitate acquisition of the best quality data for your particular animal model.

SINGLE PROBE IMAGING

1. Prepare the imaging probe according to included instructions. All probes must be injected systemically either through the retro-orbital plexus or the tail vein. For most of the probes intraperitoneal (IP) or subcutaneous (SC) injection will either not work at all or will be extremely variable and with high injection site signal.
2. Place a heating pad beneath the anesthesia induction chamber to keep the body temperature of the mice constant. Be careful not to overheat. Anesthetize the first mouse by placing it in a gas anesthesia induction chamber.
3. Remove the mouse from the induction chamber when it appears completely anesthetized, and confirm the depth of anesthesia through unresponsiveness to toe pinch.
4. Inject the appropriate volume of probe (100 - 150 µL, as per each probe's specific instructions) via the retro-orbital plexus (or tail vein) of the anesthetized mouse. Record the injection time.
5. Return the mouse to the cage for recovery and go to the next mouse for injection.
6. Repeat steps 1 - 6 until all mice are injected.
7. Imaging is performed at the suggested time(s) using a single excitation/emission filter pair optimal for the wavelength of the probe to be imaged (see table). Anesthetize mice using inhaled anesthesia and place them carefully in the appropriate orientation in the imaging system. Multiple images can be acquired with little or no concern for photobleaching of the probes.

TWO PROBE IMAGING

1. Prepare the first imaging probe as described in that probe's instructions. Either use the Probe 1 solution to solubilize Probe 2 in order to minimize injection volume or make each probe at half-volumes for mixing. [Note: Bear in mind that three of the probes (Annexin-Vivo™ 750, MMPSense® 680, and ProSense® 680) come in 10X liquid form]. Multiple specific strategies for preparation are possible, but it is ideal to keep mouse injection volumes under 250 µL.
2. Prepare and inject mice as described above.
3. Imaging should be performed as described above, but for both 680 nm and 750 nm using the appropriate excitation and emission filter pairs. Correct times for acquisition should be noted; although most of the probes are optimal for 24 h imaging, some can be imaged earlier, and some should be imaged earlier (see table). For example, Annexin-Vivo 750 imaging is optimal for most applications at 2 h, whereas ProSense 680 is optimal at 24 h. You can either image both wavelengths at both 2 and 24 h, or you can image 750 nm at 2 h and 680 nm at 24 h.
4. Multiple repeat acquisitions can be performed with little or no concern for photobleaching of the probes.

LONGITUDINAL IMAGING

1. PerkinElmer probes are well characterized with respect to tissue clearance kinetics, providing guidance for longitudinal imaging strategies. Depending on the probe, reinjection generally can be performed three to seven days following the first image acquisition.
2. OsteoSense® 680EX bone turnover imaging, however, requires a different strategy due to the very long tissue clearance kinetics. Secondary imaging time points must be performed using a pre-imaging strategy; briefly, mice should be imaged immediately prior to each additional probe injection to allow subtraction correction of additional imaging datasets.

TOXICOLOGY PROBE COCKTAIL

1. PerkinElmer recently published research showing the utility of an Annexin-Vivo/MMPSense/Transferrin-Vivo cocktail of 750 nm probes (AMT-750) in the imaging of drug-induced liver injury (Vasquez & Peterson, J Pharmacol Exp Ther 2017; 361:87-98). The Toxicology Panel of probes provides an extra vial of MMPSense 750 FAST to allow the preparation of AMT-750.
2. Prepare the AMT-750 as below:

Probe	Prepared Stock Concentration	Volume
Annexin-Vivo 750	As supplied in liquid form	0.5 mL
MMPSense 750 FAST	Prepare as 80 uM stock in PBS	0.5 mL
Transferrin-Vivo 750	Prepare as 10 uM stock in PBS	0.5 mL
		Total 1.5 mL Mouse Dose 150 µL

   The ratio of the cocktail is specifically designed to optimize liver injury signal while minimizing normal background signal in liver and kidneys.
3. AMT-750 should be injected retro-orbitally or via tail vein at 2 h or 24 h post-drug treatment. Some drugs (given as a single IP bolus of 100 - 300 mg/kg) will induce early biological changes predictive of tissue injury, whereas others require 24 h to manifest tissue biological changes. Imaging time is optimized for 24h post-AMT-750 injection (Vasquez & Peterson, 2017).

Probe Dose and Clearance Table

Part Number	Probe	Packaged Amount	Mouse Dose (25 g)	Optimal Imaging Time	Probe Clearance	Route of Metabolism/ Background Tissue(s)	ex/em wavelengths (IVIS® Spectrum)
NEV10054EX	AngioSense® 680 EX	24 nmol	2 nmol	24 h	6-7 d	Low Liver, Lung	675/720
NEV10723EX	AngioSense 680 EX Sample Size	8 nmol	2 nmol	24 h	6-7 d	Low Liver, Lung	675/720
NEV10011EX	AngioSense 750 EX	24 nmol	2 nmol	24 h	6-7 d	Low Liver, Lung	745/800
NEV11053	Annexin-Vivo 750	1 mL	100 uL	2 h	3 d	Kidneys (High), Liver	745/800
NEV10090	BombesinRSense™ 680	24 nmol	2 nmol	24 h	6-7 d	Pancreas, Kidney	675/720
NEV11000	CAT K 680 FAST	24 nmol	2 nmol	6-24 h	3 d	Kidney > Liver	675/720
NEV11112	CAT B 680 FAST	24 nmol	2 nmol	6-24 h	3 d	Salivary Glands > Liver, Kidneys	675/720
NEV11098	CAT B 750 FAST	48 nmol	4 nmol	6-24 h	3 d	Salivary Glands > Liver, Kidneys	745/800
NEV10040	FolateRSense™ 680	24 nmol	2 nmol	6 h (6-24)	6-7 d	Kidneys	675/720
NEV10645	IntegriSense™ 680	24 nmol	2 nmol	24 h	14 d	Kidneys	675/720
NEV10873	IntegriSense 750	24 nmol	2 nmol	24 h	4-6 d	Kidneys	745/800
NEV10878	IntegriSense 750 Sample Size	7 nmol	2 nmol	24 h	4-6 d	Kidneys	745/800
NEV11169	Neutrophil Elastase 680 FAST	48 nmol	4 nmol	3-6 h	2 d	Bladder > Liver, Intestines	675/720
NEV10126	MMPSense 680	20 nmol	2 nmol	24 h (24-36)	6-7 d	Liver	675/720
NEV10168	MMPSense 750 FAST	24 nmol	2 nmol	24 h (12-24)	6-7 d	Liver > Kidneys	745/800
NEV10932	MMPSense 750 FAST Sample Size	7 nmol	2 nmol	24 h (12-24)	6-7 d	Liver > Kidneys	745/800
NEV10020EX	OsteoSense 680EX	24 nmol	2 nmol	3-24 h	4 weeks	Bladder	675/720
NEV10003	ProSense 680	20 nmol	2 nmol	24 h (24-48)	6-7 d	Liver	675/720
NEV10001EX	ProSense 750 EX	24 nmol	2 nmol	24 h	6-7 d	Low Liver, Intestine	745/800
NEV10972EX	ProSense 750 EX Sample Size	8 nmol	2 nmol	24 h	6-7 d	Low Liver, Intestine	745/800
NEV11171	ProSense 750 FAST	48 nmol	4 nmol	6-24 h	3 d	Low Liver, Bladder	745/800
NEV11079	ReninSense™ 680 FAST	24 nmol	2 nmol	24 (12-24) h	4 d	Kidney, lung, liver	675/720
NEV10091	Transferrin-Vivo™ 750	24 nmol	2 nmol	24 (6-24) h	4 d	Liver, kidney	745/800
NEV11118	VivoTag™ 680XL Protein Labeling Kit	Kit: 2 x 0.25 mg	Labeling reaction	Dependent on the protein that is conjugated	Dependent on the protein that is conjugated	Dependent on the protein that is conjugated	675/720
770504	XenoLight® RediJect™ D-Luciferin	10 x 850 µL of 30 mg/mL	125 µL per 25 g mouse (or 150 µL per 30 g mouse)	10-20 min or as determined by kinetic curve*	NA	NA	NA

*See "Determining the Luciferin Kinetic Curve for Your Model" available on our website or by contacting Global Technical Support at **global.techsupport@perkinelmer.com**

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