

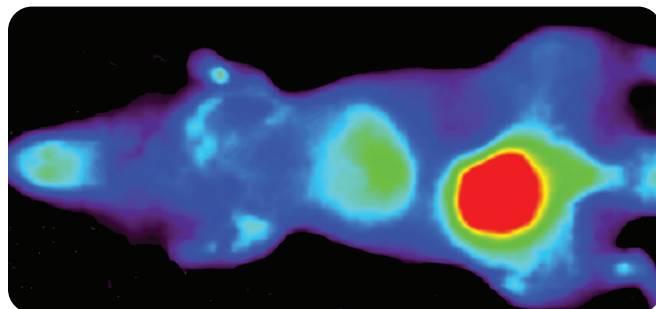
IVISense Transferrin Receptor 750 Probe (NEV10091) *In vivo* imaging protocol

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®.
- 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 IVISense™ Transferrin Receptor 750

- Each vial contains 24 nmol of IVISense Transferrin Receptor 750 in 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 Transferrin-Vivo per mouse.
- Once reconstituted with 1 x PBS, the solution is stable up to 14 days when stored at 2-8 °C 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.
- 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 IVISense Transferrin Receptor 750 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 IVISense Transferrin Receptor 750 is 24 hours. The optimal re-injection time is 96 hours, which allows for the complete clearance of the agent from the mouse.

IVIS Imaging

- If using the 2D (epifluorescence) feature on the IVIS imaging system, 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/SpectrumCT: 745 ex/800 em
 - IVIS Lumina: 745 ex/ICG em (standard Lumina filter set) or 745 ex/800 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 SpectrumCT.
- For further information on trans-illumination or FLIT please refer to tech notes under the help tab of the Living Image® software.

For more information on fluorescence imaging, see 'Best Practices for Designing an Effective *In Vivo* Fluorescence Imaging Study' available on our website.

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