Technical information	Fluorescent Imaging Reagent

Caution: For Laboratory Use. A product for research purposes only.

Aqua-ICG NHS Ester

Indocyanine green (ICG) is a near-infrared (NIR) fluorophore widely used for multiple imaging applications and in medical diagnostics.[1] ICG has a peak spectral absorption at about 800 nm and therefore possesses ideal properties for tissue fluorescent imaging. In addition, ICG has excellent optoacoustic and Short-wave infrared (SWIR II) properties (defined as light in the 0.9 – 1.7µm wavelength range).[2]

However, ICG has several significant shortcomings. Due to hydrophobic nature of the molecule. **ICG is poorly dissolved in aqueous solutions**. As the result, when conjugated to water-soluble biological molecules such as peptides and antibodies it causes precipitation of the resulting conjugates. For example, **Fig. 1A** shows solution of Her2 antibody (Trastuzumab) labeled with classical ICG-NHS ester reagent, resulting in formation of insoluble precipitate of the resulting conjugate. Another major problem comes from the fact that absorbance and fluorescence of **ICG degrades very quickly when placed in solution or biological tissues (photobleaching)**.

***SwissLumix* (Switzerland)** has recently developed a novel **Aqua-ICG** molecule where ICG dye is chemically protected with small biodegradable molecule, which is non-toxic and widely used in many drug formulations. **Aqua-ICG** obviates all the shortcomings of the original dye and previously used derivatives. While leaving ICG core structure chemically unmodified, **Aqua-ICG has significantly improved aqueous solubility and photostability properties while remaining to be completely non-toxic.** The functionalized **Aqua-ICG NHS** ester version of this compound is designed for selective and efficient labeling of any antibody, peptide, nanoparticle, or small molecule drug.

Her2 antibody labelled with **ICG NHS ester**

Her2 antibody labelled with **Aqua-ICG NHS ester**

Figure 1. Comparison of solubility and stability between Her2 antibody conjugate *of classical ICG-NHS ester and* ***Aqua-ICG*** *NHS ester conjugates*

Fig. 1B clearly demonstrates that unlike classical **ICG NHS** ester (**Fig. 1A**), conjugation of **Aqua-ICG NHS** ester to antibodies (Her2) produces stable compounds that do not form precipitate.

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shining light on biology

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1. **Aqua-ICG has significantly improved photostability properties** (2x in comparison to original ICG) and can be successfully used for imaging of biological processes both in live cells and living mice using standard ICG imaging settings. **Fig. 2A** demonstrates that the dye works really well for fluorescent imaging of cells. In this experiment **Aqua-ICG NHS** ester was conjugated to Her2 antibody and incubated for 24 hrs with 200K cells expressing Her2 receptor. **Fig. 2B** shows an example of application of this molecule in live animals, bearing Her-2 positive tumor xenografts.

2. The new **Aqua-ICG molecule possesses identical optoacoustic and SWIR II characteristics to the original ICG dye**, that have been reported to be superior to other available near-IR compounds on the market such as IRDye 800CW (Licor).

3. Aqua-ICG is also available in the form of free carboxylate (**Aqua-ICG carboxylate**) and maleimide (**Aqua-ICG maleimide).** The maleimide version of **Aqua-ICG** labels free sulfhydryl groups in molecules at physiological pH, such as cysteine residues in proteins. **Aqua-ICG PEG** Contrast Agent is another product that is designed as a non-specific imaging agent intended to exploit Enhanced Permeability and Retention (EPR) that is common in tumor vasculature.

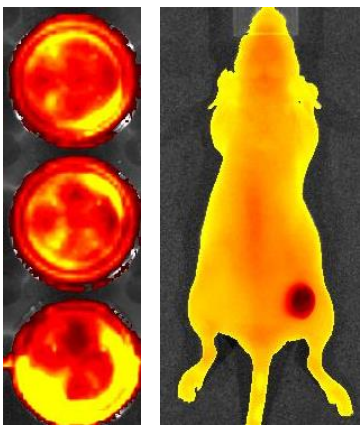

Figure 2. a) Her2 antibody conjugate *with* ***Aqua-ICG*** *NHS ester was incubated with 200K Her 2 positive cells. The image was take using IVIS spectrum (Perkin Elmer). B)* **Animals bearing Her2 positive tumor were injected with *Her2 antibody conjugate and imaged 24hrs post injection using IVIS Spectrum (Perkin Elmer)***

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