Optimally labeled fluorescent antibodies for *in vivo* animal imaging

SAIVI™ ALEXA FLUOR® ANTIBODY/PROTEIN LABELING KITS.

Stringent labeling requirements for *in vivo* animal imaging applications

The optimal fluorescent antibody conjugate for *in vitro* detection assays produces an intense fluorescent signal yet retains the binding selectivity and kinetics of the unlabeled antibody. When preparing a fluorescent antibody conjugate for *in vivo* animal imaging, however, the pharmacokinetics of the labeled probe must also be considered. In both *in vitro* and *in vivo* applications, a more heavily labeled antibody does not usually produce a better conjugate. Antibody conjugates with a very high degree of labeling (DOL) typically precipitate out of solution, bind nonspecifically, or exhibit intramolecular fluorescence quenching, making it necessary to have a less-than-maximal DOL to obtain a functional fluorescent antibody. For *in vivo* labeling experiments, the DOL of an antibody conjugate is even more severely restricted because it has significant consequences for the biodistribution and clearance of the probe. These additional constraints have led us to investigate the effects of DOL on fluorescent antibody conjugates used for *in vivo* animal imaging.

Effects of DOL on pharmacokinetics of fluorescent antibodies

To examine the pharmacokinetics of fluorescent antibodies with different DOL, we labeled anti-CEA (carcinoembryonic antigen) antibody at 1.1, 2.4, 3.9, and 6.0 dyes per antibody using the SAIVI™ Alexa Fluor® 750 Antibody/Protein Labeling Kit, which is specifically designed for creating azide-free antibody conjugates for injection. These fluorescent antibody conjugates were then injected intravenously into mice bearing LS174-T human tumor xenografts and imaged at varying times postinjection. As seen in Figure 1, the xenograft was most clearly detected using the fluorescent antibody with a DOL of 2.4. When imaged ventrally, these same mice exhibited nonspecific liver fluorescence that was more intense as the DOL of the antibody conjugates increased (Figure 2), suggesting an antibody clearance mechanism mediated by the liver.

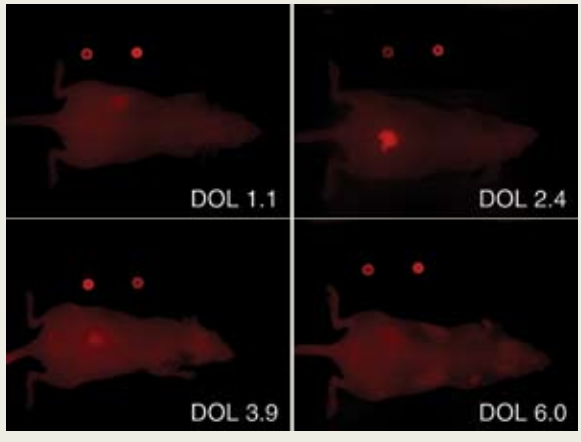

Figure 1—The fluorescence intensity of *in vivo* xenograft target labeling is not proportional to the degree of labeling (DOL) of the antibody conjugate. Anti-CEA (carcinoembryonic antigen) antibody (Zymed® Col-1 clone) was labeled with Alexa Fluor® 750 dye at different DOL (1.1, 2.4, 3.9, and 6.0) using the SAIVI™ Alexa Fluor® 750 Antibody/Protein Labeling Kit. Fifty micrograms of each of these fluorescent anti-CEA conjugates was then injected intravenously via the tail vein of athymic nude (nu/nu) mice carrying LS174-T (ATCC) human colon cancer xenografts of similar size. At 24 hours postinjection, the mice were imaged dorsally using an excitation wavelength of 735 nm and an emission range of 790–950 nm on the Maestro™ In-Vivo Imaging System (Cambridge Research & Instrumentation). The image cubes were spectrally processed using the Maestro™ software, which uses multispectral imaging technology to spectrally isolate the Alexa Fluor® 750 emission from autofluorescence in a defined area, and then quantitates it. As shown here, increasing the DOL of the fluorescent antibody above the optimal level caused a decrease in signal intensity. The two fluorescent dots at the top of each image were placed directly on the stage at the time of image collection to control for intensity levels.

This effect of DOL on pharmacokinetics is not limited to anti-CEA antibody conjugated to Alexa Fluor® 750 dye. We have observed similar results with anti-CEA antibody conjugated to Alexa Fluor® 680 dye, as well as with anti-EGFR (epidermal growth factor receptor) antibody conjugated to either Alexa Fluor® 680 dye or Alexa Fluor® 750 dye (Figure 3). The SAIVI™ Alexa Fluor® Antibody/Protein Labeling Kits—available with a choice of three different near-IR–emitting Alexa Fluor® dyes—provide a convenient method for preparing fluorescent antibody conjugates while carefully controlling the labeling using the DOL modulating reagent. By enabling you to quickly and reproducibly label and purify antibody conjugates with varying ratios of dye to protein, these kits allow you to optimize the fluorescence labeling in your *in vivo* imaging experiments. ■

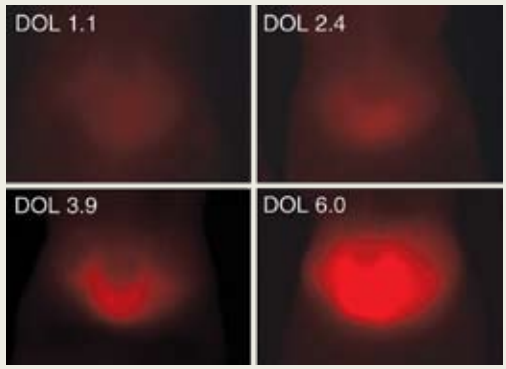

Figure 2—The fluorescence intensity of *in vivo* liver accumulation increases as the degree of labeling (DOL) of the antibody conjugate increases. The same mice shown in Figure 1 were also imaged ventrally using an excitation wavelength of 735 nm and an emission range of 790–950 nm on the Maestro™ In-Vivo Imaging System (Cambridge Research & Instrumentation). As the DOL of the fluorescent antibody increased, the signal intensity in the liver region also increased. At higher than optimal DOL, the intensity of the liver signal was greater than the intensity of the xenograft target signal (data not shown).

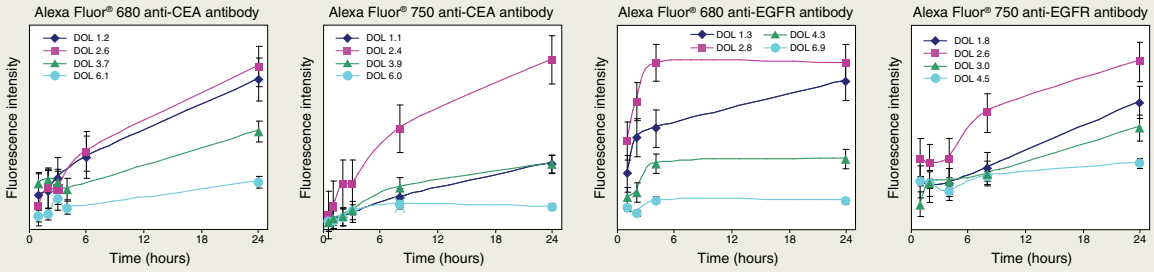

Figure 3—The fluorescence intensity of *in vivo* xenograft target labeling varies with degree of labeling (DOL) of the antibody conjugate. Two monoclonal mouse antibodies—the anti-CEA (carcinoembryonic antigen) antibody (Zymed® Col-1 clone) and the anti-EGFR (epidermal growth factor receptor) antibody (Zymed® 31G7 clone)—were conjugated to either Alexa Fluor® 680 or Alexa Fluor® 750 dyes at varying DOL using the SAIVI™ Alexa Fluor® Antibody/Protein Labeling Kits, injected into xenograft-bearing mice, and then imaged at various time points using the Maestro™ In-Vivo Imaging System (Cambridge Research & Instrumentation). In these examples, the fluorescent antibodies that produced the highest fluorescence intensity at the xenograft site had a DOL between 2 and 3.

Product	Quantity	Cat. no.
SAIVI™ Alexa Fluor® 647 Antibody/Protein 1 mg Labeling Kit *3 labelings*	1 kit	S30044
SAIVI™ Alexa Fluor® 647 Antibody/Protein 0.1 mg Labeling Kit *5 labelings*	1 kit	S30043
SAIVI™ Alexa Fluor® 680 Antibody/Protein 1 mg Labeling Kit *3 labelings*	1 kit	S30039
SAIVI™ Alexa Fluor® 680 Antibody/Protein 0.1 mg Labeling Kit *5 labelings*	1 kit	S30041
SAIVI™ Alexa Fluor® 750 Antibody/Protein 1 mg Labeling Kit *3 labelings*	1 kit	S30040
SAIVI™ Alexa Fluor® 750 Antibody/Protein 0.1 mg Labeling Kit *5 labelings*	1 kit	S30042