Service Sheet

QVQ
QUALITY IN ANTIBODIES

Yalelaan 1
3584 CL Utrecht
The Netherlands
+31 30 253 3421

www.qvquality.com
KvK: 30274082
VAT: 8215.17.168
NL88 RABO0153194936

Generation of sdAb-fluorophore conjugates

Small-sized sdAb conjugates

Single-domain antibodies (sdAb, Figure 1) are small in size (~15 kDa) which gives them several advantages in imaging applications compared to full-length antibodies.[1] Because of the small distance between epitope and label, fluorescent sdAbs have a small linkage error making them suitable for the sensitive, direct detection of binding (e.g. flow cytometry) or protein-protein interaction (e.g. BRET).[2] Furthermore, sdAbs have good tissue penetration and rapid systemic clearance *in vivo* making them valuable tools for 3D and *in vivo* imaging.[3,4]

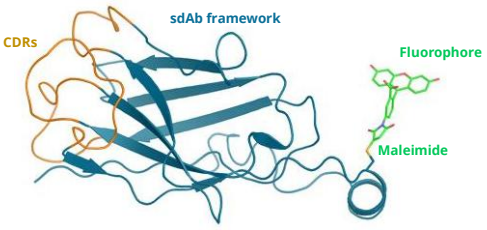

Figure 1. Structure model of sdAb-fluorophore conjugate. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-modified fluorophore (green).

Organic dyes

Organic fluorophores are small chemical molecules that emit light after excitation. A diverse range of these dyes has been developed in the last decades, featuring various excitation and emission spectra covering the visible spectrum and extending into the near-infrared range (Figure 2).[5] Using small-molecule dyes keeps the overall size of the conjugate small retaining the benefits of sdAbs for detection and imaging applications.

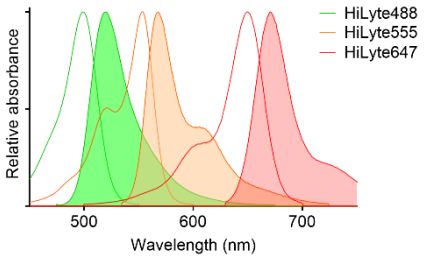

Figure 2. Spectra of HiLyte dyes. Excitation/ emission maxima: HiLyte488: 502/527 nm, HiLyte555: 552/569 nm, HiLyte647: 649/674 nm.

sdAb-fluorophore conjugation

QVQ generates sdAb-fluorophore conjugates via a Click Chemistry reaction between an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and a maleimide-modified dye. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 3).

sdAb conjugates are routinely generated with the fluorophores of the visible spectrum (ATTO- or HiLyteFluor dyes) , Figure 2) as well as the near-infrared dyes IRDye® 680RD and IRDye® 800CW. Other fluorophores can be conjugated on request.

Deliverables

- Fluorescently labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - UV-Vis spectrum
  - Percentage of free dye (SDS PAGE, fluorescent scan)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

Examples:

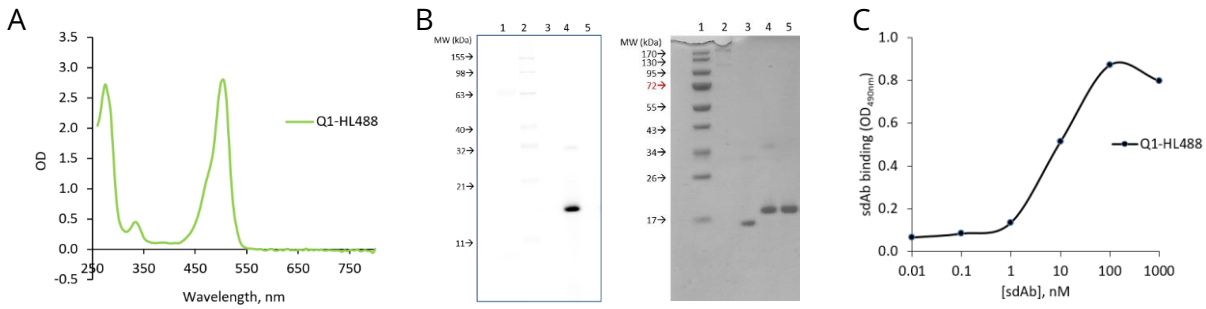

Figure 3. Example of quality control of generated sdAb-fluorophore conjugate Q1c-HL488. A) UV-Vis spectrum of sdAb-fluorophore conjugate Q1-HiLyteFluor488. B) SDS PAGE of 1) Marker, 2) Fluorescent Marker, 3) Reference sdAb (1 ug), 4) Q1c-488, and 5) unlabeled Q1c scanned for fluorescence (left) and after PageBlue staining (right). C) Binding of conjugate to recombinant HIV-encoded gp120 in ELISA. Bound sdAb was detected with rabbit-anti-VHH (QE19), followed by donkey-anti-rabbit-HRP and OPD as substrate.

References

1 Hamers-Casterman et al. (1993) Nature 363(6428), 446-448.
2 Van den Bor, Bergkamp et al (2023) Cell Rep. Methods 3(3), 100422.
3 Jeremiasse et al (2024) EMBO molecular medicine 16(7), 1495-1514.
4 Kijanka et al (2016) EJNMMI Res 6(1), 14.
5 Grimm and Lavis (2021) Nat. Methods 19, 149-158.