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-biotin conjugates

sdAb detection with biotin

Single-domain antibodies (sdAbs) are valuable detection agents due to their small size and specificity. Detection of antigen-bound sdAbs can be achieved via secondary antibodies or directly via conjugated labels.

One widely used label is biotin, a small, biological molecule that interacts with the much larger and tetrameric proteins avidin, streptavidin, and derivatives of those.[1] The interaction of avidin and biotin is one of the strongest non-covalent interactions known ($K_D \approx 10^{-15}$ M).[2] This makes the biotin-(strept)avidin complex a useful tool in various biochemical assays detecting proteins or protein-protein interactions.[3,4]

Biotinylated sdAbs (Figure 1) allow for capturing on (strept)avidin-coated surfaces and sensitive detection of sdAb binding and presence of antigens in e.g. ELISA and SPR.

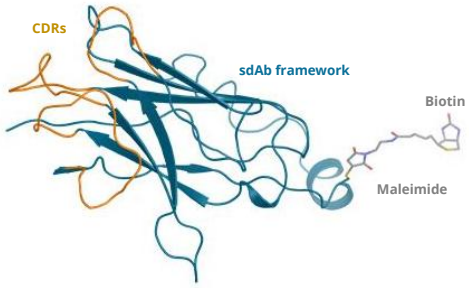

Figure 1. Structure model of sdAb-biotin conjugate. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-biotin.

sdAb-biotin conjugation

sdAb-biotin conjugates are generated via a click reaction of an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and maleimide-modified biotin. The resulting conjugate is checked for protein integrity, degree of labeling by using 4'-hydroxyazobenzene-2-carboxylic acid (HABA), and target binding in ELISA, SPR, or flow cytometry (Figure 2).

Deliverables

- Biotin-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity of the conjugate (ELISA, biotin detection)

Examples:

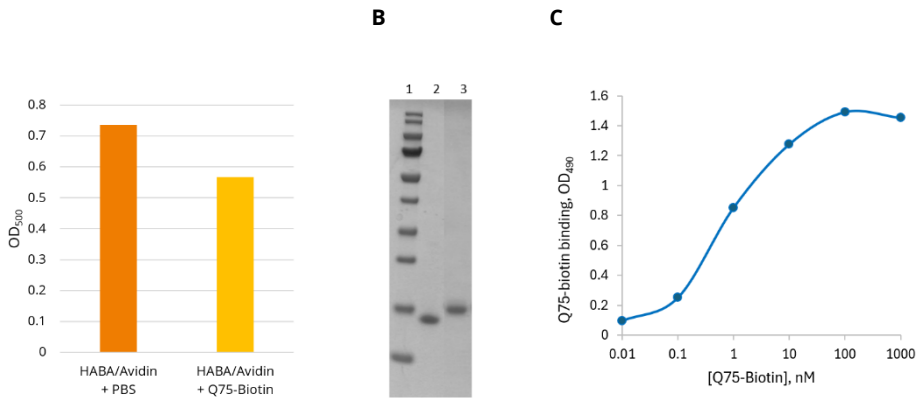

Figure 2. Example of quality control of conjugated sdAb-biotin. A) Results of HABA assay for the determination of DOL. B) SDS PAGE of 1) Marker, 2) Reference VHH (1 ug), and 3) Q75-biotin conjugate. C) sdAb-biotin binding to immobilized recombinant ITGB1 in ELISA detected with ExtrAvidin-HRP.

References:

1 Laitinen et al (2006) Cell. Mol. Life Sci. 63, 2992–3017.
2 Kuhn and Kollmann (2000) J. Am. Chem. Soc. 122, 3909-3916.
3 Ren, Han et al (2015) Chem. Commun. 51, 10403.
4 Dundas et al (2013) Appl Microbiol Biotechnol. 97(21), 9343-9353.