Service Sheet

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Generation of sdAb-HRP conjugates

Horseradish Peroxidase

Horseradish peroxidase (HRP) is a 44 kDa protein derived from horseradish roots[1]. HRP is a metalloenzyme catalyzing the oxidation of organic substrates leading to the formation of chromogenic or fluorogenic products.[2,3] It is widely used as a reporter enzyme in various stainings and assays.[4] When combined with detection probes, such as antibodies, HRP can be employed for the sensitive detection of targets, for instance using ELISA.[4]

Detecting binding of primary probes in ELISA typically involves using secondary and even tertiary antibodies. Using directly labeled sdAb-HRP (Figure 1) allows for immediate detection shortening assay time drastically. Using sdAb-HRP also allows for a straightforward assessment of sdAb-sdAb competition (Figure 2).

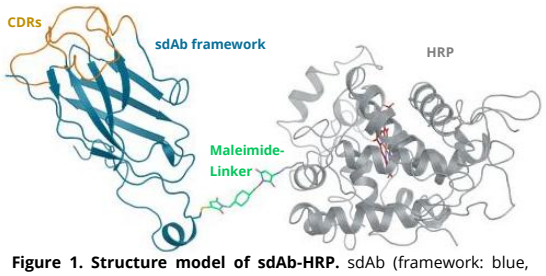


Figure 1. Structure model of sdAb-HRP. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to bifunctional linker (green) to HRP (grey).

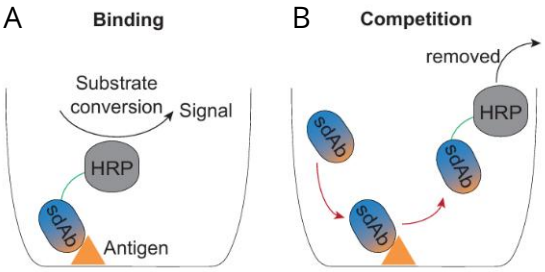


Figure 2. Illustration of sdAb-HRP in ELISA. sdAb-HRP binds to immobilized antigen. After washing away unbound protein, bound sdAb-HRP is quantified by colorimetric substrate conversion. This allows for detecting sdAb-HRP binding (A) and competition with unlabeled sdAbs (B).

sdAb-HRP conjugation

sdAb-HRP conjugates are generated by using a bifunctional linker targeting an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and a lysine of the HRP. The resulting conjugate is checked for protein integrity, degree of labeling, target binding, and HRP functionality (Figure 3).

Deliverables

- HRP-conjugated sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Percentage of free HRP (SDS PAGE, PageBlue stained)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)
  - Confirmation of HRP functionality (ELISA)

Examples:

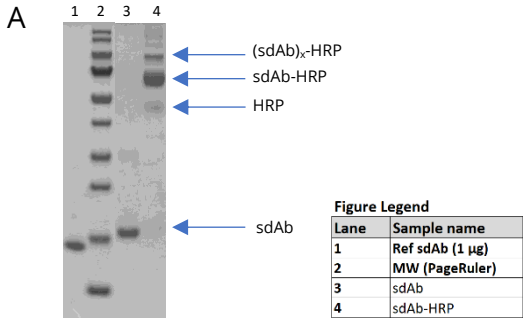
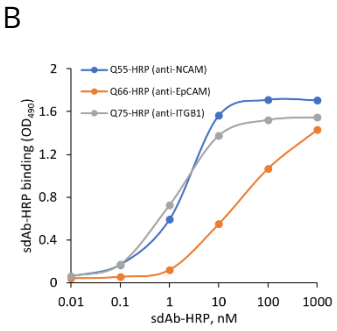


Figure Legend

Lane	Sample name
1	Ref sdAb (1 µg)
2	MW (PageRuler)
3	sdAb
4	sdAb-HRP



Example of quality control of generated sdAb-HRP conjugates. A) PageBlue stained SDS PAGE of sdAb-HRP conjugate. B) Binding of sdAb-HRP conjugates to immobilized recombinant antigens detected using OPD as substrate.

References:

1 Berglund et al. (2002) Nature 417(6887), 463–468.
2 Veitch (2004) Phytochem. 65(3), 249-259.
3 Meng et al. (2005) Anal. Biochem. 345(2), 227-236.
4 Krainer and Glieder (2015) Appl. Microbiol. Biotechnol 99, 1611-1625.