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1. Description

This product is for research use only.

Components	10 mL Protein Preparation Buffer 50 µL Reducing Reagent (10× concentrated) 1 × Ni-NTA Column 1 mL Ni-NTA Elution Buffer 2 × Protein Concentrator (including two dedicated collection tubes) 3 × 2 mL Collection Tube
Capacity	For one conjugation reaction of 100 µg of VHH antibody.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the box label.

1.1 Principle of the Oligonucleotide Conjugation Kit, ThioLink

The Oligonucleotide Conjugation Kit, ThioLink enables the easy and efficient generation of VHH antibody–oligonucleotide conjugates in less than 5 hours. This kit has been optimized for the conjugation of 100 µg of protein (i.e. VHH antibodies) to proprietary ssDNA-oligonucleotides, provided by Miltenyi Biotec (see section 1.3).

The conjugation of VHH antibody to oligonucleotides is achieved in several consecutive steps (see Figure 1), starting with the chemical activation of the VHH antibody by reducing the accessible thiol group (–SH group, a functional side chain of the amino acid cysteine). Then follows the ligation of maleimide-activated oligonucleotides to the thiol group. After ligation, excessive oligonucleotides are removed through washing and purification steps and a terminal protein concentration step ensures that the VHH antibody–oligo conjugate is available in the optimal concentration for downstream applications.

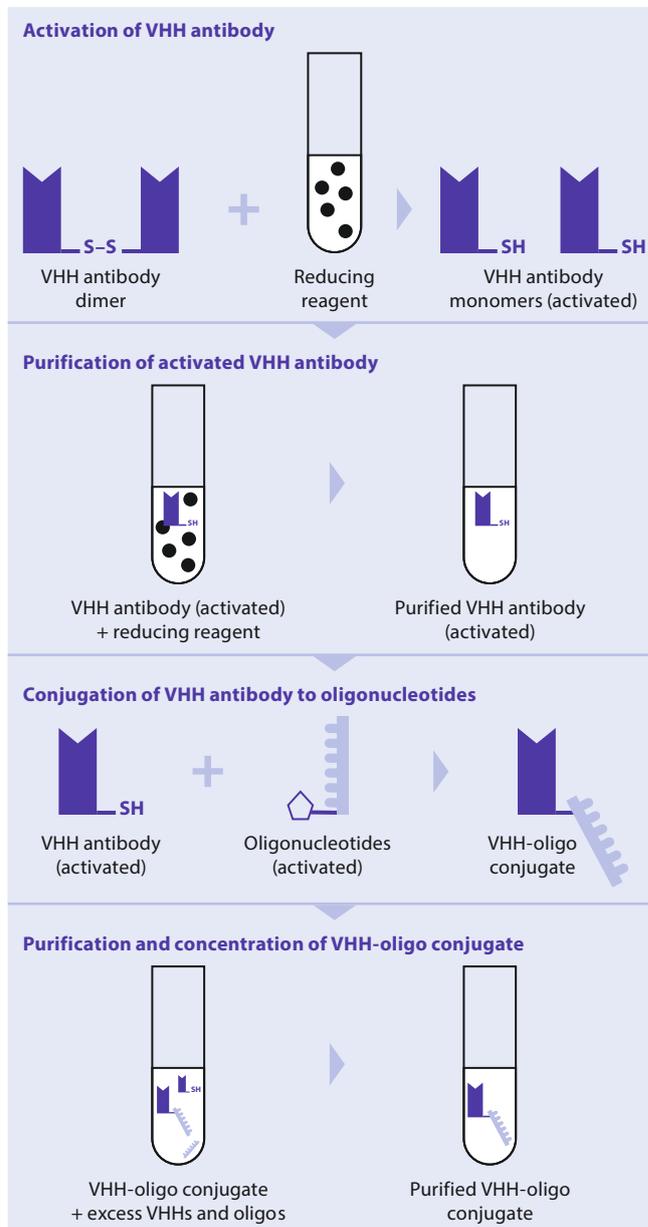


Figure 1: Principle of Oligonucleotide Conjugation Kit, ThioLink for the conjugation of VHH antibody with an accessible Thiol group (–SH).

1.2 Applications

The oligo-conjugated VHH antibodies, can be selectively and reversibly attached to biosensor chips of the MACS Matchmaker focal molography platform via DNA-directed immobilization (DDI). Protein-loaded chips are then available for biosensing applications, such as protein-protein interaction analysis.

Alternatively, the oligo-conjugated VHH antibodies can also be used in imaging applications where the oligonucleotide serves as a barcode for DNA-mediated signal amplification.

1.3 Reagent and instrument requirements

- 100 µg of VHH antibody at a concentration of 1 mg/mL, free from carrier proteins with solvent-accessible thiol groups or disulfide bridges, as these will interfere with the conjugation reaction.
 - ▲ **Note:** Strictly avoid thiol containing components or reducing compounds in the formulation, such as mercaptoethanol, DTT, TCEP, etc. If required, perform buffer exchange prior to starting the conjugation process.
- ssDNA Oligonucleotides, 10–80 nt long, containing a 5'-terminal maleimide group, reconstituted at 100 µM in nuclease-free water, phosphate-buffered saline (PBS, pH 7.4), or Protein Preparation Buffer.
 - ▲ **Note:** The kit is compatible with Oligo Adapters Maleimide specifically designed for the use on the MACS Matchmaker focal molography platform.
- Centrifuge with fixed-angle rotor that can accommodate 2 mL centrifuge tubes.
- Thermomixer that can accommodate 2 mL centrifuge tubes.

2. Protocol

2.1 Activation of VHH antibody

The VHH antibody is activated by reducing its accessible thiol group, allowing it to later react with the maleimide-activated oligonucleotide.

1. Briefly spin down the vial containing 10× Reducing Agent to collect any liquid from the sides of the tube.
2. Add 450 µL of Protein Preparation Buffer to the tube with the 10x Reducing Agent.
3. Mix the solution thoroughly by pipetting up and down.
4. Add 50 µL of 1× diluted reducing agent from step 3 to the solution containing 100 µg of VHH antibody and mix thoroughly by pipetting up and down.
5. Incubate the mixture in a thermomixer at +25 °C and 500 rpm for 1 hour.

▲ **Note:** VHH antibody may precipitate when vortex or centrifuge at high rpm.

2.2 Washing and recovery of activated VHH antibody

After the activation of VHH antibody, any excess reducing agent needs to be removed to prevent interference with the conjugation step.

2.2.1 Wash the Protein Concentrator

1. Add 500 µL of Protein Preparation Buffer to the Protein Concentrator, placed in its dedicated collection tube. Cap the tube.
 - ▲ **Note:** Always ensure the cap strap and one membrane panel of the Protein Concentrator tube faces the center of the rotor.
2. Centrifuge at 14,000 × g for 10 minutes.
3. Discard the flow-through.

2.2.2 Perform buffer exchange

4. Adjust the volume of the activated VHH antibody from step 5 of section 2.1 to the final volume of 450 µL with Protein Preparation Buffer.
5. Add the VHH antibody solution to the Protein Concentrator.
6. Centrifuge at 14,000 × g for 10 minutes.
7. Discard the flow-through.
8. Add 450 µL of Protein Preparation Buffer to the Protein Concentrator.
9. Centrifuge at 14,000 × g for 10 minutes.
10. Discard the flow-through.
11. Repeat the steps 8, 9, and 10.

2.2.3 Recover the VHH antibody

12. Invert the Protein Concentrator into a fresh dedicated collection tube.
13. Centrifuge at 1,000 × g for 2 minutes to collect the concentrated VHH antibody.
 - ▲ **Note:** Approximately 50 µL of VHH antibody at approx. 2 mg/mL is collected in the tube.

2.3 Conjugation of activated VHH antibody to oligonucleotide

The activated VHH antibody is conjugated to an oligonucleotide via thiol-maleimide reaction.

▲ The oligonucleotide should be reconstituted at 100 µM in nuclease-free water, phosphate-buffered saline (PBS, pH 7.4), or Protein Preparation Buffer.

1. Add 300 µL of maleimide-oligonucleotide (100 µM) to the VHH antibody collected in step 12 of section 2.2.
2. Incubate the mixture in a thermomixer at +25 °C and 500 rpm for 1 hour.

2.4 Removal of free oligonucleotides

In the next steps, the conjugated VHH antibody is purified from excess of free oligonucleotides.

2.4.1 Wash the Ni-NTA Column

1. Remove the bottom closure and loosen the cap of the Ni-NTA Column.
 - ▲ **Note:** Do not discard the bottom closure.
2. Place the column in a fresh 2 mL Collection Tube and centrifuge at 700 × g for 2 minutes to remove storage solution. Discard the flow-through.
3. Add 400 µL of Protein Preparation Buffer to the column. Centrifuge at 700 × g for 2 minutes to equilibrate the column. Discard the flow-through.
4. Repeat step 3 for two additional times.

2.4.2 Purify the VHH antibody

5. Put the bottom closure to the Ni-NTA Column. Place the column back into the 2 mL Collection Tube used in steps 1–4, section 2.4.1.

6. Apply the VHH antibody sample from section 2.3 to the center of the column.
7. Incubate the mixture in a thermomixer at +25 °C and 500 rpm for 30 minutes.
8. Remove the bottom closure from the Ni-NTA Column. Centrifuge at 700 × g for 2 minutes. Discard the flow-through.
9. Add 200 µL of Protein Preparation Buffer to the column. Centrifuge at 700 × g for 2 minutes. Discard the flow-through.
10. Repeat step 9, section 2.4 for four additional times.
11. Place the column in a fresh 2 mL Collection Tube.
12. Add 200 µL of Ni-NTA Elution Buffer to the column. Centrifuge at 700 × g for 2 minutes. **Collect** the flow-through.
13. To fully recover the VHH antibody conjugate, repeat the step 12, section 2.4 for two additional times. Combine the eluted fractions in the same Collection Tube.

2.5 Removal of imidazole

After the elution of the VHH antibody conjugate from the Ni-NTA Column, the solution contains imidazole that might interfere with the concentration measurements or downstream applications.

2.5.1 Wash the Protein Concentrator

1. Add 500 µL of Protein Preparation Buffer to the Protein Concentrator, placed in its dedicated collection tube. Cap the tube.
2. Centrifuge at 14,000 × g for 10 minutes.
3. Discard the flow-through.

2.5.2 Perform buffer exchange

4. Add 300 µL out of 600 µL of the VHH antibody conjugate solution from step 13, section 2.4 to the freshly washed Protein Concentrator from step 3, section 2.5.
5. Centrifuge at 14,000 × g for 10 minutes.
6. Discard the flow-through.
7. Repeat the steps 4 to 6, section 2.5 by adding the remaining 300 µL of the VHH antibody conjugate solution.
8. Add 450 µL of Protein Preparation Buffer to the Protein Concentrator and centrifuge at 14,000 × g for 10 minutes. Discard the flow-through.
9. Repeat step 8, section 2.5.2.

2.5.3 Recover the VHH antibody conjugate

10. Invert the Protein Concentrator into a fresh dedicated collection tube.
11. Centrifuge for 2 minutes at 1,000 × g to collect the concentrated VHH antibody conjugate. The Collection Tube now contains the purified VHH antibody conjugate which can be used for immobilization on the biosensor chip of the MACS Matchmaker instrument.

▲ Approximately 50 µL of VHH antibody conjugate at approx. 1.5 mg/mL (100 µM) are collected in the tube. Approximately 25% of the VHH antibody conjugate may be lost during the purification steps.

▲ The collected sample may contain remnants of non-conjugated VHH antibody and traces of free oligonucleotides. For applications where the presence of these residues may impact results, an additional chromatographic purification step is recommended.

▲ Aliquot the conjugated protein to convenient volumes and store at +4 °C for up to 3 months.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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