Miltenyi Biotec

Oligonucleotide Conjugation Kit, AminoLink

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1. Description

This product is for research use only.

Components
10 mL Protein Preparation Buffer
100 µL Linker Reconstitution Medium
200 µL Oligonucleotide Capturing Slurry
100 µg Bifunctional Linker
1 × Desalting Column
1 × Spin Column (including a cap-free collection tube)
1 × Protein Concentrator (including two dedicated collection tubes)
3 × 2 mL Collection Tube

Capacity For one conjugation reaction of 100 µg of protein (15–300 kDa).

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, AminoLink

The Oligonucleotide Conjugation Kit, AminoLink has been designed for the efficient conjugation of proteins to proprietary ssDNA oligonucleotides used in focal molography applications on the MACS® Matchmaker instrument. The labeled protein can be immobilized on the biosensor chips to analyze biomolecular interaction with other molecules or proteins.

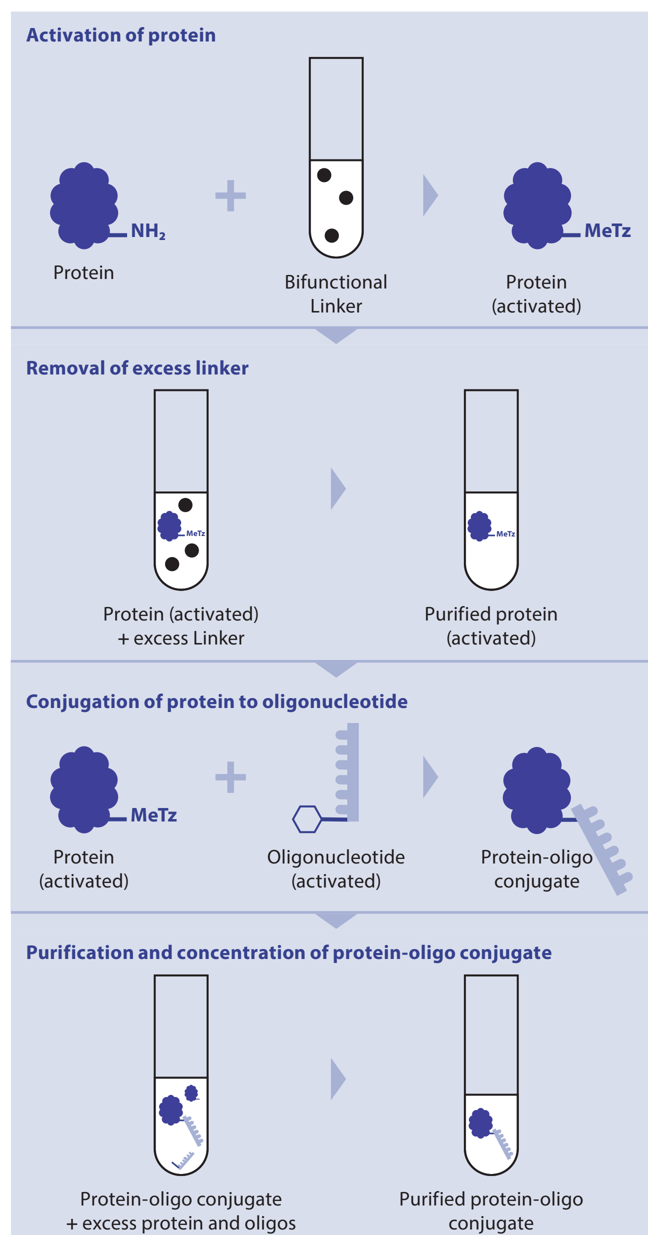

Figure 1: Principle of Oligonucleotide Conjugation Kit, AminoLink for the conjugation of the protein activated with an accessible methyltetrazine moiety (–MeTz).

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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The Oligonucleotide Conjugation Kit, AminoLink enables the easy and efficient generation of protein-oligonucleotide conjugates in less than 4 hours. This kit has been optimized for the conjugation of 100 µg of protein (15–300 kDa) to proprietary ssDNA-oligonucleotides, provided by Miltenyi Biotec (see section 1.3).

The conjugation of proteins to oligonucleotides is achieved in several consecutive steps, starting with the chemical activation of the protein with the help of a bifunctional linker (**see Figure 1**). The linker reacts with an amine (NH_2) group on the protein and activates it with a bioorthogonal methyltetrazine (MeTz) moiety. Once modified, the protein can be conjugated to DNA-oligonucleotides containing a trans-cyclooctene (TCO) group. After ligation, excessive oligonucleotides are removed through washing and purification steps. The conjugation protocol allows a flexible protein-to-oligonucleotide ratio from 1:1 to 1:5, depending on the downstream applications.

1.2 Applications

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

1.3 Reagent and instrument requirements

- 100 µg of protein (15–300 kDa) at a concentration of 1–5 mg/mL, free from carrier proteins such as Bovine Serum Albumin (BSA) or other extraneous proteins, as these will interfere with the conjugation reaction.

  ▲**Note:** Make sure the protein solution is suitable for the amine-reactive labeling method (see the table in Protocol section 2.1). If it doesn't meet any of the listed requirements, change the buffer as described in section 2.1 before starting the conjugation.

- ssDNA Oligonucleotides, 10–80 nt long, containing a terminal trans-cyclooctene (TCO) 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 TCO 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 Optional step: Protein concentration and /or buffer exchange

This step is required if the protein concentration is less than 1 mg/mL or if the protein formulation is not compatible with the requirements listed in the table:

Protein formulation	Requirement
pH	pH 7– 8
Amine free buffer (e.g. PBS)	allowed
Non-buffering salts (e.g. sodium chloride)	allowed
Chelating agents (e.g. EDTA)	allowed
Sugars	allowed
Glycerol	allowed ≤ 40 % (v/v)
Sodium azide	allowed ≤ 0.05 % (w/v)
Tris	not allowed
Glycine	not allowed
Histidine	not allowed
Primary Amines	not allowed

If the protein is lyophilized, reconstitute it to a concentration of 1 mg/mL using Protein Preparation Buffer. Then proceed to section 2.2.

2.1.1 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.

2.1.2 Concentrate the protein and/or perform buffer exchange

4. Adjust the volume of the solution containing 100 µg of protein to the final volume of 450 µL with Protein Preparation Buffer.
5. Add the protein 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 and centrifuge at 14,000 × g for 10 minutes.

   ▲**Note:** Repeat step 8 multiple times to ensure that amine-containing components (e.g. Glycine, TRIS) are removed to the levels that do not interfere with the conjugation process:

   Number of exchanges = $\log_{10}$ (concentration to remove in µM)

   For example, for a 20 mM Tris buffer, the buffer exchange should be performed for $\log_{10}(20{,}000)$ = 3.3 rounded up to 4 times, to sufficiently reduce the concentration of the amine-containing component.

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2.1.3 Recover the protein

9. Invert the Protein Concentrator into a fresh dedicated collection tube.

10. Centrifuge at 1,000 × g for 2 minutes to collect the concentrated protein.

    ▲ **Note:** Approximately 50 µl of protein sample at a concentration of approximately 2 mg/mL is collected in the tube.

2.2 Protein activation

The protein is activated with the bifunctional linker, allowing it to later undergo bioorthogonal conjugation with the oligonucleotide.

2.2.1 Reconstitute the Bifunctional Linker

1. Add 10 µL of Linker Reconstitution Medium to the Bifunctional Linker. Use a pipette to vigorously mix until the red pellet is completely dissolved.

2. Add 2.0 mL of Protein Preparation Buffer to the Bifunctional Linker. Vortex the tube for at least 5 seconds, then spin down the solution to collect any liquid from the sides of the tube.

2.2.2 Activate the protein

3. Add the appropriate volume of linker to the solution containing 100 µg of protein. Refer to formula and table below for detailed guidance on selecting the appropriate volume of linker depending on the molecular weight of the protein and the desired conjugation ratio:

$$\text{Volume of linker } (\mu L) = \frac{\text{Number of oligonucleotides per protein}}{\text{Molecular weight of protein (kDa)}} \times 1000$$

Molecular weight of the protein, kDa	Conjugation ratio (protein: oligonucleotide)		
	1:1	1:2	1:5
15	70 µL	–	–
50	20 µL	40 µL	100 µL
100	10 µL	20 µL	50 µL
150	7 µL	14 µL	35 µL

▲ **Note:** The conjugation ratio represents a statistical average, meaning that while most proteins will have the desired number of oligonucleotide strands attached, individual proteins may carry varying numbers of oligonucleotides per molecule.

4. Incubate the mixture in a thermomixer at +25 °C and 800 rpm for 1 hour.

2.3 Removal of excess bifunctional linker

After protein activation, any excess linker needs to be removed to prevent side reactions during the oligonucleotide conjugation step.

2.3.1 Wash the Desalting Column

1. Remove the bottom closure and loosen the cap of Desalting Column. Place the column in a fresh 2 mL Collection Tube.

2. Centrifuge at 1,500 × g for 1 minutes to remove storage solution.

3. Discard the flow-through.

4. Place a mark on the side of the Desalting Column where the compacted resin is slanted upward. Place the Desalting Column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

5. Add 300 µL of Protein Preparation Buffer to the column. Centrifuge at 1,500 × g for 1 minute to equilibrate the column.

6. Discard the flow-through.

7. Repeat step 5 for two additional times. Discard the flow-through each time.

2.3.2 Purify the protein

8. Adjust the volume of the protein to a final volume of 130 µL with Protein Preparation Buffer.

9. Place the Desalting Column into a fresh 2 mL Collection Tube and slowly apply the protein sample to the center of the compact resin.

10. Centrifuge at 1,500 × g for 2 minutes to collect the sample.

2.4 Conjugation of protein to oligonucleotides

In the next steps the activated protein is conjugated with the TCO-activated oligonucleotides via bioorthogonal click chemistry.

▲ **Note:** The oligonucleotides should be reconstituted at 100 µM in nuclease-free water, Protein Preparation Buffer, or any recommended buffer.

1. Add the appropriate volume of oligonucleotide to the protein collected in step 10 of section 2.3.2. For detailed guidance on selecting the appropriate volume of oligonucleotide depending on the molecular weight of the protein and the desired conjugation ratio, refer either to the formula or the table below:

$$\text{Volume of oligonucleotide } (\mu L) = \frac{\text{Number of oligonucleotides per protein}}{\text{Molecular weight of protein (kDa)}} \times 1500$$

Molecular weight of the protein, kDa	Conjugation ratio (protein: oligonucleotide)		
	1:1	1:2	1:5
15	100 µL	–	–
50	30 µL	60 µL	150 µL
100	15 µL	30 µL	75 µL
150	10 µL	20 µL	50 µL

2. Incubate the mixture in a thermomixer at +25 °C and 800 rpm for 1 hour.

2.5. Removal of free oligonucleotides

The conjugated protein is purified from excess of free oligonucleotides.

2.5.1 Wash the Oligonucleotide Capturing Slurry

1. Briefly vortex the vial containing the Oligonucleotide Capturing Slurry to ensure the slurry is evenly suspended before use.

2. Add 200 µL of Oligonucleotide Capturing Slurry to the spin column.

3. Place the column in a cap-free collection tube and centrifuge at 700 × g for 2 minutes to remove storage solution.

4. Discard the flow-through.

5. Add 200 µL of Protein Preparation Buffer to the column. Centrifuge at 700 × g for 2 minutes to equilibrate the column.

6. Discard the flow-through.

7. Repeat step 5 for two additional times. Discard the flow-through each time.

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2.5.2 Purify the protein

8. Adjust the volume of the protein sample to a final volume of 200 µL with Protein Preparation Buffer.
9. Put the bottom closure to the spin column. Place column in a fresh 2 mL Collection Tube.
10. Slowly apply the sample to the center of the Oligonucleotide Capturing Slurry.
11. Incubate the mixture in a thermomixer at +25 °C and 800 rpm for 30 minutes.
12. Remove the bottom closure from the spin column. Centrifuge at 700 × g for 2 minutes to collect the sample in the 2 mL Collection Tube.
13. Add 200 µL of Protein Preparation Buffer to the column. Centrifuge at 700 × g for 2 minutes to fully recover the protein conjugate. The 2 mL Collection Tube now contains the purified protein conjugate which can be used for immobilization on the biosensor chip of the MACS Matchmaker instrument.

▲**Note:** The final concentration of the conjugate may vary depending on the nature of the protein and the conjugation parameters used. If precise concentration determination is necessary, assess the concentration using an appropriate assay, following the manufacturer's instructions.

▲**Note:** The collected sample may contain remnants of non-conjugated protein 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.

▲**Note:** If long-term storage or a specific application requires a concentrated protein sample, an optional protein concentration step is recommended.

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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