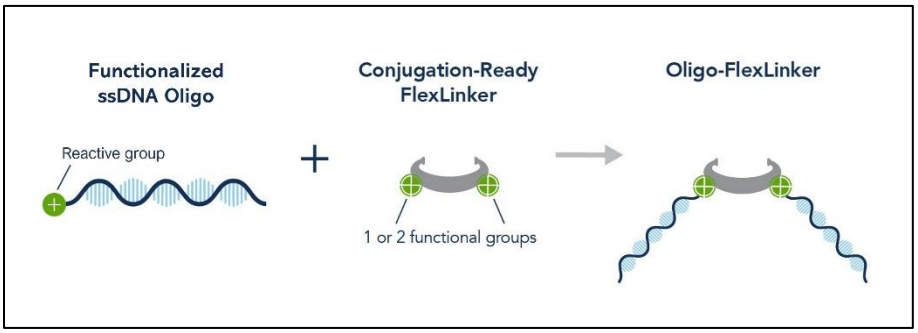
FlexAble Oligo-Ready Antibody Labeling Kit

How does it work?

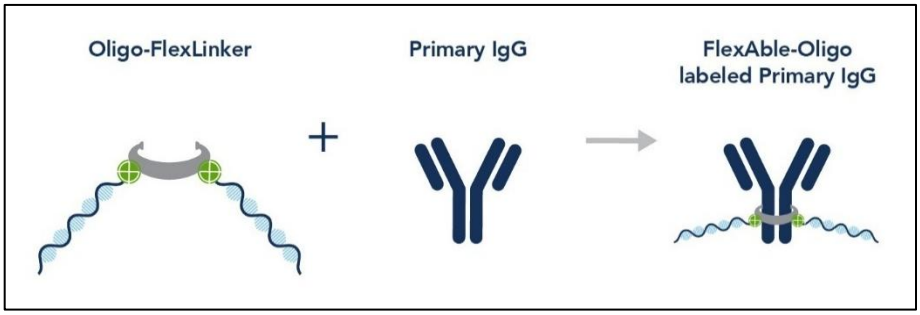
Part 1. Preparation of Oligo-FlexLinkers



DBCO-functionalized ssDNA oligo reacts to DBCO-ready FlexLinkers, carrying 1 or 2 azide groups for Azide-DBCO click conjugation, to generate Oligo-FlexLinkers. The protocol includes a clean-up step to remove excess free oligo.

- *1 Kit provides enough conjugation-ready FlexLinker for conjugation of up to 3 different Oligos to FlexLinker (scalable from 3x 33 µg – 1x 100 µg).*
- *Oligo-FlexLinkers are stable and can be stored at -20°C for up to 6 months.*

Part 2. Labeling of Antibodies with Oligo-FlexLinkers



*Oligo-FlexLinkers are used to label primary antibodies in a simple **15 min** protocol with minimal hands-on time.*

- *1 Kit yields sufficient Oligo-FlexLinker to label a total of up to 100 µg of primary antibody.*
- *The minimum amount of antibody required per label-reaction is 0.5 µg.*

General Notes

FlexAble Oligo-Ready Kits provide a quick and efficient method for labeling antibodies with oligos using FlexAble Antibody Labeling Technology. It uses high-affinity, Fc-specific labeling reagents called **FlexLinkers, which carry 1 or 2 azide groups that react with DBCO-oligos** through click chemistry. Following conjugation, Oligo-FlexLinker conjugates enable site-specific and high-affinity labeling of antibodies, ensuring consistent and reliable performance.

- One kit provides enough FlexLinker to label a total of 100 µg of antibody. Reaction volumes can be proportionally adjusted. You can perform multiple smaller reactions or one larger reaction, depending on your experimental needs
  The protocol describes the preparation of sufficient Oligo-FlexLinker to label up to 33 µg of primary antibody and uses one-third of the provided conjugation-ready FlexLinker.

- We do not recommend using less for preparation of each Oligo-FlexLinker, as this might significantly impact the yield through loss of sample during the preparation.

- The protocol contains a clean-up step for removal of excess DNA.

- The intermediate product, the Oligo-FlexLinker, can be stored at -20°C (for up to 6 months) or used directly to label the desired amount of primary antibody.

- You can label as little as 0.5 µg of primary antibody per reaction, enabling you to label up to 200 different primary antibodies with one kit or scale up according to your needs (labeling up to 100 µg of one antibody).

- Please note that the kit does not contain all reagents required for the protocol.
  The following items must be sourced from your lab or other vendors:

Reagent	Amount	Used for
DBCO-Oligo	5 or 10 nmol (for 1 or 2 azide respectively) for 1/3 of the kit (scale up to your needs; examples see next page)	Conjugation to FlexLinker
Ni-NTA Agarose (e.g.. Protino Ni-NTA Agarose Macherey-Nagel, 745400.25)	100 µl for 1/3 of the kit (scale up to your needs)	Removal of free DNA, Oligo-FlexLinker binds to Ni^{2+} via His-tag
PBS (Nuclease free)	5 ml per Oligo-FlexLinker conjugation	Washing steps
Imidazole (1M stock)	1 ml per Oligo-FlexLinker conjugation	Elution Buffer
Desalting columns (e.g., Zeba™ Spin Desalting 2 mL Columns, Thermo, 89890)	1x per Oligo-FlexLinker conjugation	Imidazole removal

Part 1.1. Preparation of Oligo-FlexLinkers

This part describes the conjugation of the FlexLinker with a DBCO-functionalized oligo. For Amine oligos, please start with the optional part on page 7.

Note: *This part describes the preparation of enough Oligo-FlexLinker to label 33 µg of primary antibody. Volumes can be scaled proportionally to yield sufficient Oligo-FlexLinker for labeling of 100 µg. Examples for upscaling this part are given in the box below. Once the Oligo-FlexLinker is prepared, as little as 0.5 µg of primary antibody is required per labeling reaction (see Part 2).*

	FlexLinker (2 µg/µl)		Oligo (100 µM)	
I want to label...	for 1 & 2 Oligos		1 Oligo	2 Oligos
33 µg of antibody *	16 µl		50 µl (5 nmol)	100 µl (10 nmol)
50 µg of antibody	25 µl	+	75 µl (7.5 nmol)	150 µl (15 nmol)
100 µg of antibody **	48 µl		150 µl (15 nmol)	300 µl (30 nmol)

* *16 µl FlexLinker + 50 µl Oligo will yield sufficient Oligo-FlexLinker-conjugate to label a total of 33 µg of primary antibody (or as little as 0.5 µg of antibody in one reaction).*

** *48 µl FlexLinker + 150 µl Oligo will yield sufficient Oligo-FlexLinker-conjugate to label a total of 100 µg of primary antibody (or as little as 0.5 µg of antibody in one reaction).*

Required Materials

Included with Kit

- FlexAble 1x or 2x Azide (2 mg/mL, use 16 µL for one conjugation out of 50 µL total)

Not Included

- DBCO-Oligo; 100 µM; 5 nmol (1xAzide) or 10 nmol (2xAzide)
  (dissolved in 1x PBS pH 7.4)

Procedure

1. Mix 16 µL FlexLinker (1x or 2x Azide) with DBCO-oligo (50 µL or 100 µL, respectively). The final molar ratio of oligo:FlexLinker is approximately 10:1.

2. Incubate overnight at room temperature.

3. Proceed to purification as described in part 1.2. - Removal of free oligo.

Part 1.2. Removal of free Oligo

This part describes the removal of excess oligo from Oligo-FlexLinkers.

The purification includes the following 2 sections:

- Ni-NTA purification to remove free unconjugated DNA oligo.
- Buffer exchange to remove imidazole from previous elution step.

Note: To scale up this clean-up step, only increase bead and elution volumes. Do not increase PBS volumes for washing steps, as the column has a volume limit. The amount of washing steps can be increased.

Required Materials

Included with Kit

- Spin columns

Not included

- Oligo-FlexLinker conjugate, produced as described in part 1.1; (66 - 116 µL)
- Protino Ni-NTA Agarose (Macherey-Nagel, 745400.25, 50 µL) or equivalent
- Zeba™ Spin Desalting 2 mL Columns (Thermo, 89890, 1 pcs) or equivalent
- Binding & Washing Buffer: PBS
- Ni-NTA Elution Buffer: Mix 1 mL PBS with 1 mL of 1 M imidazole (final conc. = 500 mM Imidazole)

Procedure

Preparation of Ni-NTA resin

1. Add 100 µL Ni-NTA Agarose (slurry) to a ChromoTek spin column (open bottom plug) placed in a 2 mL Eppendorf tube.

2. Spin at 1000 x g for 1 min and discard flowthrough.

3. Add 500 µL PBS to beads.

4. Spin at 1000 x g for 1 min and discard flowthrough.

5. Repeat steps 3 & 4.

Ni-NTA Purification – Removal of free oligo

1. Close bottom plug of the ChromoTek spin column and add the Oligo-FlexLinker-conjugate (66 – 116 µL) to beads. Rotate slowly end-over-end for 30 min at room temperature.

2. Open bottom plug of spin column and place it in a 2 mL Eppendorf tube.

3. Spin at 1000 x g for 1 min and discard flowthrough (contains free oligos).

4. Add 500 µL PBS to beads.

5. Spin at 1000 x g for 1 min and discard flowthrough.

6. Repeat steps 4 & 5.

7. Close the spin column with bottom plug and add 100 µL Ni-NTA Elution Buffer to the settled beads. Rotate end-over-end for 5 min at room temperature.

8. Transfer the spin column into a fresh 2 mL Eppendorf tube, remove bottom plug, and spin at 1000 x g for 1 min.

9. Repeat steps 7 & 8 to recover all samples (use same tube for both elution steps).

Buffer Exchange

1. Exchange buffer, e.g., with a Zeba™ Spin Desalting column to PBS, according to manufacturer's manual.

Note: Purified Oligo-FlexLinker-conjugates can be stored at -20°C for up to 6 months.

Part 2: Labeling of Primary Antibody with Oligo-FlexLinker

Required Materials

Included with Kit

- FlexQuencher (IgG species and subtype specific)
- FlexBuffer

Not included

- Purified Oligo-FlexLinker-conjugate (prepared in part 1)
- Primary antibody (IgG species and subtype matching the FlexAble Oligo-Ready Kit)

Procedure

1. Estimate protein concentration via BCA enhanced assay (recommended), SDS-PAGE (load reference amounts side by side with 5 - 10 µl of Oligo-FlexLinker-conjugate), or Qubit assay.
2. Mix 0.25 µg of Oligo-FlexLinker (should ideally equal 2 – 3 µl) with 0.5 µg of primary antibody. If the total volume is less than 8 µL, adjust the volume to 8 µL with FlexBuffer and incubate for 5 min at room temperature.
3. Add 2 µL FlexQuencher, mix, and incubate for 5 min at room temperature.
4. The antibody is now ready to use.

Note: The volumes in this part refer to a single FlexAble reaction, which labels 0.5 µg of primary antibody. Volumes / amounts can be scaled according to how much antibody you want to label with the prepared Oligo-FlexLinker-conjugate. To do that, scale linearly with amount of primary antibody. The Oligo-FlexLinker volume needed per reaction depends on efficiency and yield of the preparation.

Labeling with Oligo-FlexLinkers only takes 15 min, minimal hands-on time, and requires only a minimal amount of antibody, meaning you can easily prepare the exact amount of antibody needed immediately before use. However, if storage of FlexAble-labeled antibody is required, optimal storage conditions should be determined for each individual primary antibody and may vary. Normally, storage of a few days at +4°C is possible.

Optional: DBCO-PEG4 Labeling of Oligo

This part describes the functionalization of amine-modified oligos with a DBCO group. Omit this step if starting with a DBCO-oligo directly.

Required Materials

Not included with Kit:

- Amine-modified DNA oligo (HPLC Purified), 10 nmol
- 10X Borate buffered saline pH 7.6 (BBS): 0.5 M borate, 1.5 M NaCl, pH 7.6.
  Dilution of this buffer to 1X concentration results in a pH of 8.5. (See table on next page.)
- Dimethyl sulfoxide (DMSO)
- 100 mM DBCO-PEG4-NHS in DMSO (BroadPharm, BP-22288 or equivalent)
- 1.0 M Tris-HCl pH 8.0
- DNA purification columns
  (e.g., Amersham MicroSpin G-25 Columns, Cytiva, 27-5325-01, or equivalent)
- Nuclease-free water

Procedure

** Scale up or down according to the desired amount of FlexAble labeling.*

Time required: 30 min. Prepare immediately before proceeding to Part 1 of the protocol.

Conjugation

1. Dissolve the amine-modified oligo in nuclease-free water to obtain a concentration of 250 µM.
2. Transfer 40 µl oligo (10 nmol) to a DNase/RNase-free tube.
3. Add 5 µL 10X BBS (10% of final volume), mix 10 times.
4. Add 2.5 µl of 100 mM DBCO-PEG4-NHS in DMSO, mix 10 times.
5. Incubate the reaction at room temperature for 15 minutes.
   Note: The mixture turns slightly cloudy after adding DBCO-PEG4-NHS and becomes clear after a few minutes.

6. Add second aliquot (2.5 µl) and react for an additional 15 min.

   Note: The solution turns slightly turbid or cloudy after the second addition, but this does not affect conjugation efficiency or purification of the modified oligo.

7. Add 2.0 µL of 1.0 M Tris pH 8.0 (final concentration ~40 mM) and incubate at room temperature for 5 min to quench residual NHS groups.
8. For purification of the conjugated oligo, follow the manufacturer's protocol for Amersham MicroSpin G-25 Columns (or equivalent).

   Note: Amersham MicroSpin G-25 columns are designed for oligos greater than 10 bases in length.

Preparation of 10X Borate Buffer Saline (BBS)

1. Add 100 mL Milli-Q water to a 500-mL beaker.
2. Add 75 mL 5M NaCl.
3. Weigh 7.73 grams of boric acid and dissolve in the solution under stirring.
4. Adjust pH to 7.6 with 2 M NaOH.
5. Adjust the volume to 250 mL with Milli-Q water and recalibrate the pH to 7.6 (with 2 M HCl & 2 M NaOH).
6. To prepare 1X BBS solution, take 4 mL of the 10X BBS solution and dilute it to 40 mL with Milli-Q water.
7. Adjust pH to 8.5.