ZymeBright™ Site-Specific DBCO-Ready Antibody Conjugation Kit (2x Azide)

Description

ZymeBright™ Site-Specific DBCO-Ready Antibody Conjugation Kit (2x Azide) is enzymatic method for the conjugation of any DBCO-functionalized conjugate, such as oligonucleotides, fluorophores, enzymes, beads, or bioactive molecules, to the Fc region of Rabbit IgG, Rat IgG1 & IgG2a, Human IgG1-4, or Mouse IgG1 & IgG3. The expected degree of labeling (F/P ratio) of antibodies conjugated with this kit is 2.

Kit Contents (Cat No. PK30100)

Component	Cat No.	Amount
Antibody Modifier Reagent (Vial A)	PK3007X-A	5 µL
Lyophilized Linker (2x Azide) (Vial B)	PK30100-B	100 µg
Lyophilized PTGase (Vial C)	PK3007X-C	3 mg (provided in excess)
Conjugation Buffer (Vial D)	PK3007X-D	200 µL
Lyophilized Quenching Reagent (Vial E)	PK3007X-E	5mg
0.6 mL Low Adhesion Tube	PK3007X-F	2 tubes

General Guidelines:

Storage: Store at -20°C for up to 1 year. After opening, store at 2-8°C. Protect from light and moisture. Reagents must be used within 1 week of opening.

Required Materials:

Orbital Thermal Mixer
Pipettes
Sterile PBS
EDTA
DBCO-modified conjugate
Desalting column or ultracentrifugation tube
Ultrapure water (DNase/RNase free)
Ultra-micro spectrophotometer
Method for conjugated antibody purification (see Step 16)

Prior to Beginning:

Antibody should be at 1 mg/mL in compatible buffer. Please refer to the table for buffer component compatibility.

The entire conjugation protocol should be performed in sterile conditions to avoid contamination. If the conjugate is a fluorescent molecule, protect the conjugate and conjugated antibody from light for the duration of the protocol and during storage.

Spin down Vials A, C, D, and E. 10-15 minutes prior to beginning, bring the Lyophilized Linker (2x Azide) (Vial B) and Conjugation Buffer (Vial D) to room temperature. Store Quenching Reagent (Vial E) at 2-8°C. Preheat the orbital thermal mixer to 37°C.

On day 2, bring Quenching Reagent (Vial E) to room temperature 10-15 minutes prior to beginning.

Buffer Compatibility

Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
Antibody concentration	0.5-2 mg/mL
pH	6.5-8.0
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
BSA	Yes
Sodium Azide	Yes
Chelating agents (e.g. EDTA)	Yes
Glycerol	<10%
Sugars	Yes
Gelatin	Unknown
Tris	Yes
Glycine	No
Thiomersal / Thimerosal	Yes
Merthiolate	Yes
Proclin	Yes
Borate buffer	Yes
Primary amines (e.g. lysine, peptide or ethanolamine)	No
Heavy metal ions (e.g. Zn^{2+}, Cu^{2+}, Mn^{2+}, or Fe^{3+})	No

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Protocol

Day 1: Antibody Modification

1. Add 100 µL antibody (at 1 mg/mL) to a provided 0.6 mL low adhesion tube.
2. Add 2 µL Antibody Modifier Reagent (Vial A) to antibody in 0.6 mL low adhesion tube. Pipette for 10-15 seconds to fully mix.
3. Incubate in a 3 mm orbital thermal mixer at 37°C, 800 rpm for 30 minutes. If using a 2 mm orbital thermal mixer, mix at 1000 rpm. *Note: Avoid prolonged incubation of the antibody with the Modifier Reagent.*

Prepare Lyophilized Linker and PTGase

4. Add 100 µL sterile PBS to Vial B: Lyophilized Linker. Pipette gently for 10-15 seconds to fully dissolve Lyophilized Linker.
5. Pipette entire contents of Vial B into the other 0.6 mL low adhesion tube.
6. Add 600 µL sterile PBS to Vial C: Lyophilized PTGase and pipette for 10-15 seconds to fully dissolve Lyophilized PTGase. *Note: PTGase should be used within 1 hour of dissolving to avoid a decrease in enzymatic activity and thereby a reduction of conjugation efficiency.*

Antibody Conjugation

7. Add 8 µL dissolved Lyophilized Linker (Vial B), 20 µL PTGase solution (Vial C) and 47 µL Conjugation Buffer (Vial D) to the 0.6 mL low adhesion tube from step 5. Pipette for 20 seconds to mix well.
8. Incubate in a 3 mm orbital thermal mixer at 37°C, 800 rpm for 18-24 hours. If using a 2 mm orbit thermal mixer, mix at 1000 rpm.

Day 2: Quench Conjugation Reaction

9. Add 100 µL sterile PBS to Vial E: Lyophilized Quenching Reagent. Pipette for 10-15 seconds to fully dissolve.
10. Add 83 µL Quencher Reagent Solution to the 0.6 mL low adhesion tube from step 7. Pipette for 10-15 seconds to mix.
11. If not immediately proceeding to the next step, incubate at room temperature in the dark for 30 minutes. *Note: Antibody-Linker can be conjugated immediately, placed in 2-8°C for short-term storage, or at -20°C for long-term storage.*

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12. After the Antibody-Linker conjugation reaction is quenched, perform a buffer exchange using a G25 desalting column or ultrafiltration tube to remove excess Linker-azide. Use at least two column volumes of sterile PBS + 2 mM EDTA buffer to equilibrate the G25 desalting column or ultrafiltration tube. Desalt the antibody reaction solution in the 0.6 mL low adhesion tube from step 11 and replace the antibody buffer.
13. Measure antibody concentration using an ultra-micro spectrophotometer.

Preparation of DBCO-modified Conjugate

14. Dissolve the DBCO-modified Conjugate in ultrapure water free of DNase/RNase. *Note: For oligonucleotide conjugation, dissolved DBCO-modified oligonucleotide can be used immediately, placed in 2-8°C for short-term storage, or at -20°C for long-term storage.*

CLICK Chemistry

15. Add the DBCO-Conjugate solution slowly to the Antibody-Linker solution at a molar ratio of Antibody-Linker complex to DBCO-Conjugate of 1:3-1:6.
16. If necessary, adjust volume to 100-300 uL using sterile PBS + 2 mM EDTA. Maintain an antibody concentration > 0.2 mg/mL for efficient Click chemistry. Gently pipette or vortex to avoid air bubbles. Incubate at room temperature (25°C) for 22h.

Day 3: Antibody Purification

17. To remove unbound conjugate, appropriate methods can be used, such as Proteintech's CleanAb Kits for removing unconjugated oligonucleotides (Cat No. CK001 or CK003), or other purification methods such as G25 desalting column, dialysis, ultrafiltration, Protein A, Protein G, and ion exchange chromatography.

Conjugated Antibody Storage

18. For short-term use, store conjugated antibody in PBS at -20°C. For long-term storage, store in 30% glycerol and a small amount of BSA if compatible with subsequent experiments. *Note: A small amount of precipitation in the final conjugated antibody solution will occasionally occur and will not affect antibo dy performance.*