ADC021-EN.01

ADC Conjugation Kit (Exatecan, 200μg, for human IgG4&mouse lgG)

【Catalog Number】ADC-P021

【Size】This kit is designed to conjugate 200 μg antibody.

Please read this manual carefully before performing the experiment.

For research use only, not for use in diagnostic or therapeutic procedures.

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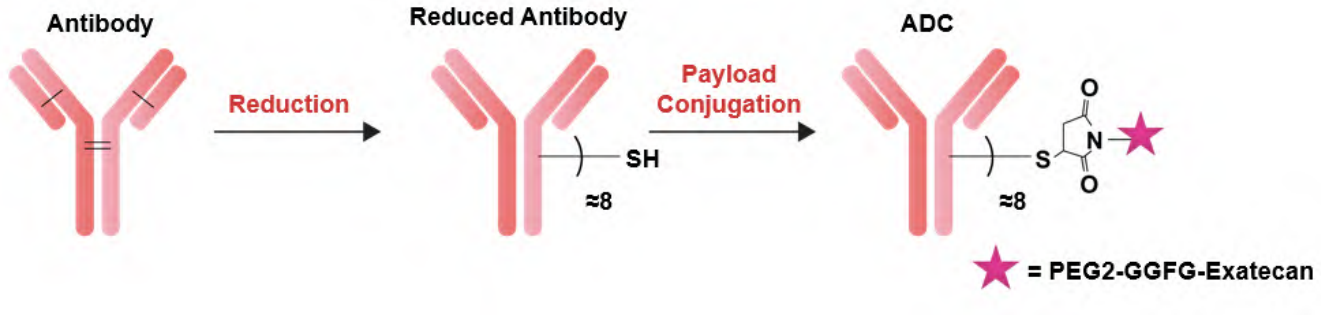
Content

【Intended Use】

The ADC Conjugation Kit is designed for conjugation of human IgG4 or mouse lgG with the cytotoxic payload Exatecan through thiol and maleimide reaction chemistry. The ADC products can be used in cellular or animal experiments for the screening of suitable ADC candidates. It is for research use only (RUO).

【Principle】

Antibody-drug conjugates (ADCs) deliver potent cytotoxic drugs specifically to tumor cells, thereby enhancing antitumor efficacy and reducing the adverse systemic effects of the cytotoxic drugs. This kit is developed based on interchain cysteine conjugation technology: the interchain disulfide bonds of antibodies can be reduced to generate endogenous interchain cysteine residues, and payload drugs with maleimide functional groups can specifically conjugate to these cysteine residues to form structurally stable ADCs. Relying on this classic conjugation strategy, the kit enables a rapid and controllable ADC preparation process that takes only 3 hours in total, achieving a conjugation efficiency greater than 90% and yielding a product with over 90% purity. The resulting ADC conjugates exhibit significant cytotoxic activity, providing a reliable experimental tool for ADC drug screening and mechanism research.



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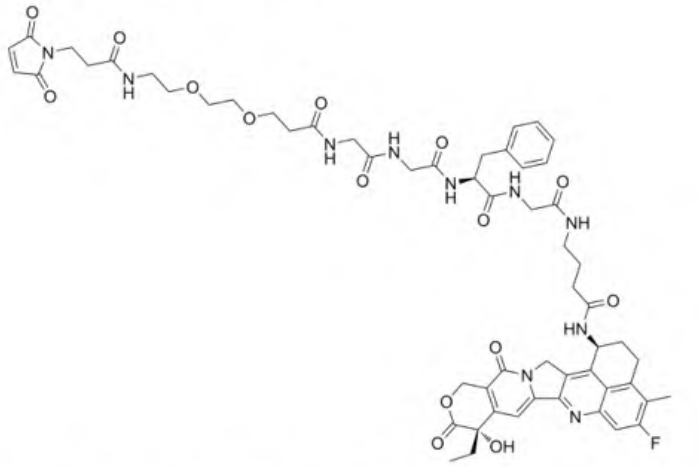
Payload information:



Name: Mal-PEG2-Gly-Gly-Phe-Gly-Exatecan

CAS: 1599439-54-9

MW: 1149.18

Formula: C57H65FN10O15

【Materials Provided】

Table 1

Catalog	ID	Components	Size (1 kit)	Storage
Box A ADC-P021	C01	Reducing reagent-01	1 vial	-20°C
	C02	Reducing reagent-02	1 vial	-20°C
	C03	Exatecan	1 vial	-20°C
	C04	Quencher reagent	1 vial	-20°C

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Table 2

Catalog	ID	Components	Size (1 kit)	Storage
Box B ADC-P021-1	Buffer01	1xPBS (pH7.2-7.4), 11%Trehalose	5 mL	4℃
	Desalting column	Desalting Spin column, 0.5mL	1 per	4℃

【Storage】

Box A: This product remains stable when stored at -20℃ for 24 months. Please ensure use prior to the expiration date indicated on the packaging.

Box B: This product remains stable when stored at 2-8℃ for 24 months. Please ensure use prior to the expiration date indicated on the packaging.

【Unsupplied Reagents and Instruments】

Thermostatic shaking incubator

Vortex mixer

Tubes: 1.5-2mL, 15mL

Fixed angle rotor centrifuge

ddH2O

UV/VIS Spectrophotometer

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【Precautions】

1. For research use only, not for use in diagnostic procedures.
2. Please use the kit within the shelf life.
3. Components of different kits and different batches of kits should not be mixed;
4. This kit is used for the conjugation of purified antibodies. Antibodies in ascites, serum, hybridoma or tissue culture may affect the conjugation result.
5. This kit is designed to conjugate human IgG1 or mouse lgG subtype antibody. Using other antibody subtypes may affect the final coupling effect. The antibody to be conjugated is provided by the user.

【Conjugation Protocol】

1. Preparation of antibody solution

1.1 Prepare the antibody at 2 mg/mL in 1×PBS buffer (pH 7.2-7.4).

Note: *Keep the antibody solution away from cysteine, glutathione or other free sulfhydryl substances, as well as BSA, gelatin or other protective proteins.*

2. Antibody Reduction

2.1 Take out each component in Box A and equilibrate to room temperature. Perform a brief centrifugation to pellet the powder at the bottom of the tube.

2.2 Add the 100 μL prepared antibody solution into the **Reducing reagent tube** (**The components corresponding to different antibody species and subtypes are shown in Table 3**), and mix them well by repeatedly pipetting or vortexing the vial for a few seconds.

2.3 Briefly centrifuge the sample tube to prevent the protein solution from adhering to the tube wall

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and to eliminate air bubbles.

2.4 Shake the reaction mixture at 100 rpm at 37 °C for 1 hour.

Note: *If the shaking incubator is unavailable, please mix the mixture using a vortex mixer every* 0.5 *hours.*

Table 3

species and subtypes of antibody	ID	Component
Mouse lgG1/2a/2b	C01	Reducing reagent-01
Human lgG4	C02	Reducing reagent-02

3. Antibody Conjugation

3.1 Equilibrate the reduced antibody solution (from step2.4) at room temperature for 5 minutes, then transfer it to the **C03-Exatecan** tube, and repeatedly pipette to dissolve the powder completely.

Note: *Ensure that the powder is completely dissolved. If the powder cannot dissolve immediately, let it stand for 2-5 minutes, and mix it again.*

3.2 Briefly centrifuge the sample tube to prevent the protein solution from adhering to the tube wall and to eliminate air bubbles.

3.3 Shake the reaction mixture at 100 rpm at room temperature (23-28 °C) for 1 hour.

4. Reaction Quenching

4.1 Add 50 µL deionized water to dissolve component **C04-Quencher reagent**, and mix it well.

4.2 The reaction mixture is quenched by the addition of 4 µL C04 solution, mix it well and keep it

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at room temperature for 0.5 hours.

5. Purification and Buffer exchange

5.1 Prepare the Buffer01 (1xPBS (pH7.2-7.4), 11%Trehalose) and the Desalting Spin column, 0.5 mL.

5.2 Remove the column's bottom closure and loosen the cap (do not remove the cap).

5.3 Place the column in a 2.0mL collection tube. Centrifuge in a centrifuge with a fixed angle rotor at 700 × g for 1 minute to remove storage solution.

5.4 Place a mark on the side of the column where the compacted resin is slanted upward. Place the column in the microcentrifuge with the mark facing outward in all subsequent centrifugation steps.

5.5 Carefully add 300µL of Buffer01 dropwise to the center of the resin bed surface without disturbing the settled resin. Centrifuge at 700 × g for 1 minute to remove buffer.

5.6 Repeat Step 5.5 once, discarding buffer from the collection tube.

5.7 Carefully add 300µL of Buffer01 to the center of the resin bed surface. Centrifuge at 700 × g for **2 minutes** to remove buffer.

5.8 Place the column in a new collection tube, remove cap and gently apply conjugated sample solution to the center of the resin bed surface.

5.9 Centrifuge at 700 × g for **2 minutes** to collect the sample. Discard the desalting column after use.

Note1: *The purification of ADC is based on the principle of size exclusion chromatography. Therefore, after applying your sample to the desalting column, it should be centrifuged as soon as possible to ensure thorough purification.*

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Note2: *This Kit provides a* 1x*PBS (pH* 7.2-7.4*) buffer with 11% Trehalose for the preservation of conjugates. The buffer is suitable for sample preservation of freezing or lyophilization, and does not affect the Hydrophobic Interaction Chromatography (HIC) analysis and cell viability test of ADCs. If your conjugates are used for Reverse Phase Chromatography (RP-HPLC) or Mass Spectrometry (MS) analysis, please replace the buffer with* 1x*PBS (pH* 7.2-7.4*), without trehalose.*

6. Concentration Determination for ADC

The Measure the UV absorbance of the conjugate at 280 nm (A280) and 370nm (A370) using a UV spectrometer to determine the concentration of the ADC, and calculate the concentration using the following formulas:

$$\boldsymbol{Concentration\ of\ ADC} = \frac{\text{A280-(A370}\times\text{0.3)}}{1.4} \times \boldsymbol{dilution\ factor}$$

Note: *For most mammalian antibodies (such as immunoglobulin), the absorption value of* 1 mg/mL *IgG at* 280 *nm is* 1.4*. You can also use your own antibody extinction coefficient instead of the default parameters.*

7. Drug-Antibody Ratio (DAR) Determination for ADC

7.1 The DAR of your ADCs could be roughly calculated by measuring the absorption values of UV 280 nm (A280) and 370 nm (A370) using the following formula. The DAR calculation using this formula is for reference purpose only.

$$\boldsymbol{DAR} = \frac{\boldsymbol{A370 \times 210000}}{\boldsymbol{20800 \times \{A280\text{-}(A370 \times 0.3)\}}}$$

7.2 The precise DAR of ADCs must be detected using HIC, RP-HPLC or liquid chromatography-mass spectrometry (LC-MS) analysis.

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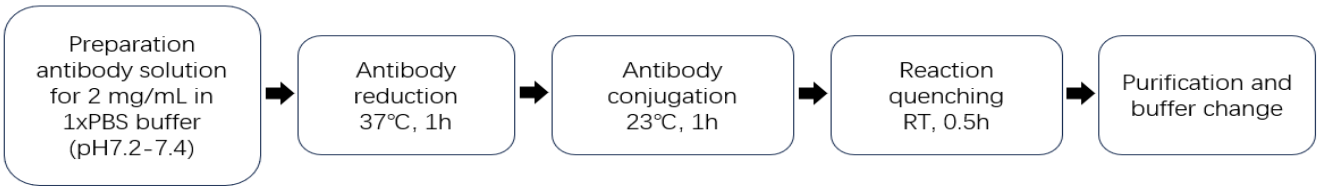
【Storage of ADC】

The optimal storage conditions should be determined based on your antibody stability. Generally, it is recommended to store the conjugate at -80℃ for long-term storage, and repeated freeze-thaw cycles should be avoided. For short-term use, the conjugate may be stored at 4℃ for up to several weeks.

【Quick Guild】

Overview of the conjugation process



【Typical Data】

For each experiment, the specific results may vary depending on different laboratories, testers, or instruments. The following data is for reference only.

The ADC was prepared using the ADC Conjugation Kit (Exatecan, for human IgG4&mouse lgG). For more detailed data, please refer to the product details webpage.

Figure 1: HIC-HPLC analysis of mouse IgG1 subtype antibody and ADC.

Figure 2: SEC-HPLC analysis of mouse IgG1 subtype antibody and ADC.

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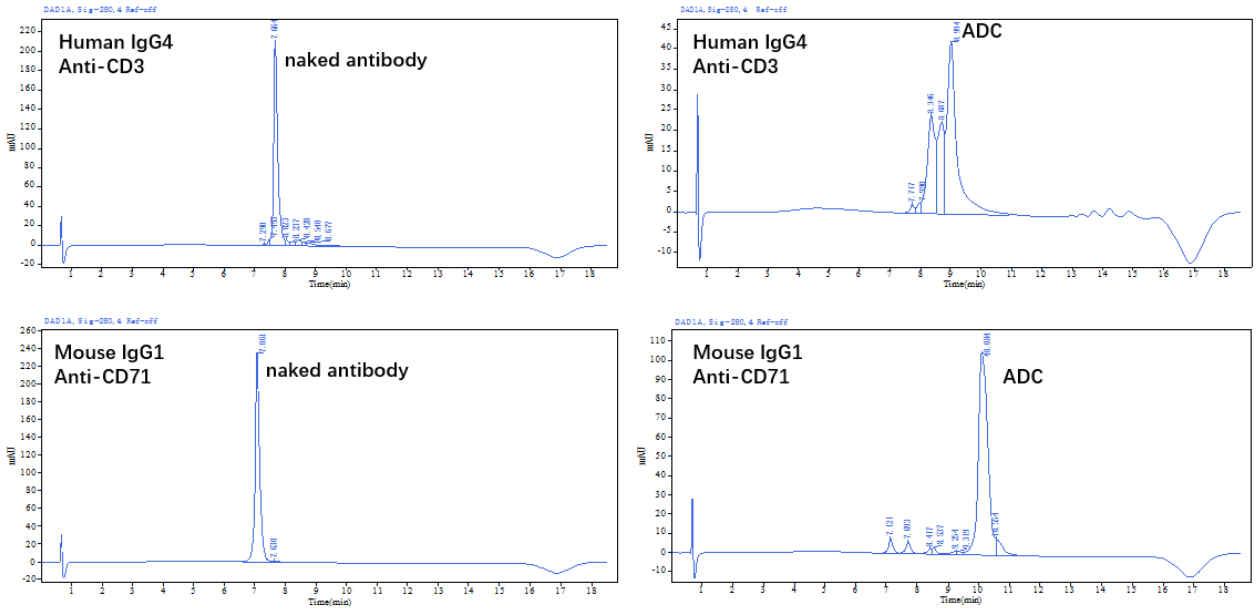

Figure 1. HIC-HPLC

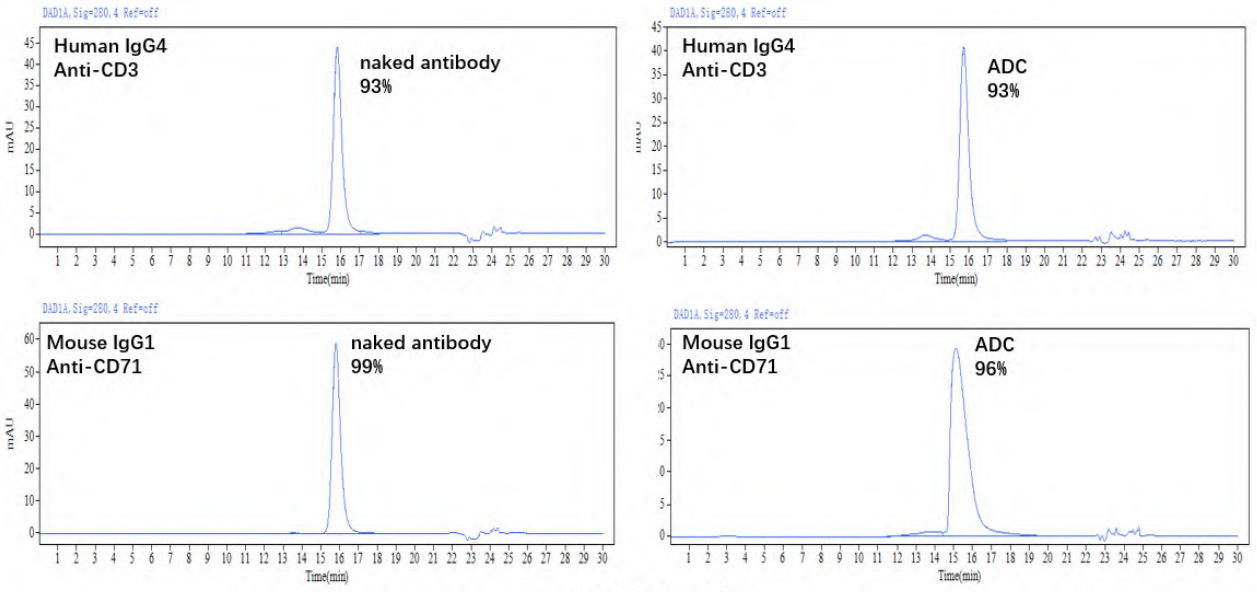

Figure 2. SEC-HPLC

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