Antibody MC-VC-PAB-Doxorubicin Conjugation Kits

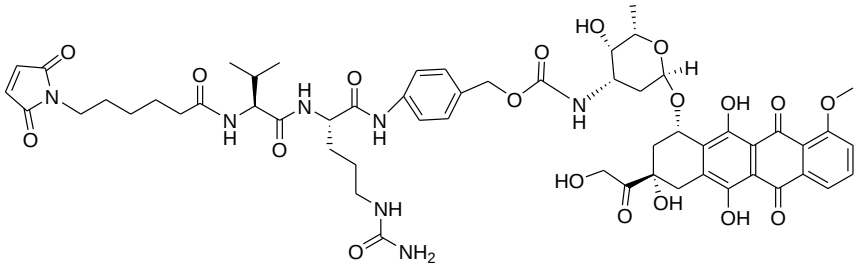
Components

Component		Product size		Storage condition
		BP-50254	BP-50255	
		1 x 1 mg	3 x 1 mg	
A	MC-VC-PAB-Doxorubicin	1 vial	3 vials	-20 °C
B	Reaction Buffer	10 ml	30 ml	4-8 °C
C	DMSO	1 ml	1 ml	Room temp.
D	TCEP	1 x 160 µg	3 x 160 µg	-20 °C
E	5x Storage Buffer	1.5 ml	1.5 ml	4-8 °C
F	Protein concentrator	1	3	Room temp.

Note:
- The kits are shipped with blue ice.

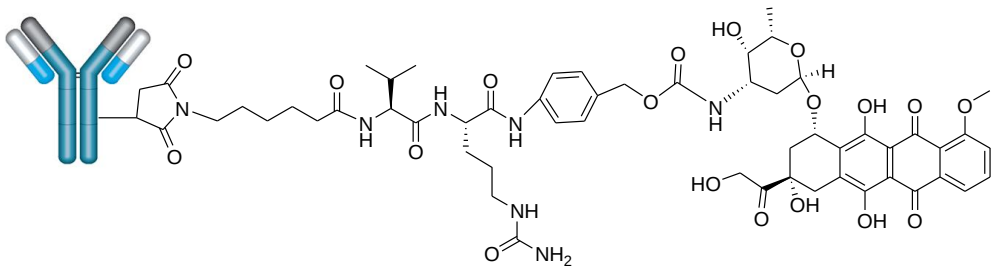
Overview

This MC-VC-PAB-Doxorubicin Conjugation Kit is designed to conjugate 1 mg of antibody with the chemotherapeutic drug, Doxorubicin via standard malemide/thiol chemistry. The linker structure (shown below) is also composed of a cleavable val-cit dipeptide, which enhances drug molecule release to the cell. The Doxorubicin is a type of chemotherapy drug called an anthracycline. It slows or stops the growth of cancer cells by blocking an enzyme called topo isomerase 2. Cancer cells need this enzyme to divide and grow. Doxorubicin is in the anthracycline and antitumor antibiotic family of medications. It works in part by interfering with the function of DNA. The Doxorubicin is very toxic to use as a drug on its own, and induce serious side effects. So, it's usually delivery a drug delivery systems to reduce the toxicity, and also attached to a monoclonal antibody (mAb) that recognizes a specific marker on cancer cells. The mAb directs Doxorubicin to the cancer cells for specific kill the cancer cells.



MC-VC-PAB-Doxorubicin
CAS No.: 159857-70-2
MW: 1142.2

Kit Features:
- Protocol: easy to follow, to conjugate 1 mg of IgG with Doxorubicin with minimum exposure to the chemotherapeutic drug
- Fast process: <1 hr hands-on time, 6 hrs for whole process.
- Convient: All reagents and supplies included for preparation purification, and storage
- DAR: average 3-8
- Effieient: More than 99% conjugated products by SEC and is free of any unreacted drugs



Antibody-VC-PAB-Doxorubicin conjugates

Technical considerations

Before you begin, prepare the antibody solution at a preferred concentration of 2 mg/ml.

Note

- If the current antibody concentation is different, adjust it to 2 mg/mL.
- The antibody should be dissolved in reaction buffer or 1X phosphate buffered saline (PBS) pH 7.2-7.5 with 1mM EDTA. If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, with EDTA, or use Amicon Ultra-0.5, Ultracel-10 Membrane, 10K MWCO (Cat # UFC501008 from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.
- Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
- The conjugation efficiency is significantly reduced if the antibody concentration is less than 1 mg/ml. For optimal labeling efficiency a final antibody concentration range of 1-5 mg/ml is recommended.

Conjugation Experimental Protocol

1. Add antibody stock to a vial of TCEP (component D), mix well, and incubate at 37 °C for 1-2 hours.
2. Reconstitute a vial of MC-VC-PAB-Doxorubicin (component A) with 100 µl of DMSO, mix to dissolve well.
3. Add all of the antibody mixture from step 2 to the vial of reconstituted MC-VC-PAB-Doxorubicin, and mix. incubate with rotation, at room temperature for 1 hour.
4. Remove excess MC-VC-PAB-Doxorubicin reagent by protein concentrator by following the instruction for desalting.

MWCO Filtration

Note: this step is to remove excess drug reagent and other small molecular side products.

a. Pre-wash the membrane by spinning with ~400 µL of DI water for 3 mins at 14,000 x g. Discard solution in upper and lower chambers.
b. Dilute the reaction mixture with buffer until the DMSO concentration is below 5% v/v.
c. Transfer the reaction mixture to the MWCO filter.
d. Spin for 15 mins at 14,000 x g. Discard solution in lower chamber.
e. Dilute solution in upper chamber with buffer.
f. Repeat steps d-e 3-6 times.
g. Transfer the solution from the upper chamber to fresh vial for final conjugation.
h. (Optional) Wash vial with DI water to maximize recovery.
i. Transfer the wash solution to the filtered solution (recovered in step g).

Drug-to-Antibody Ratio (DAR) Estimate

- Obtain absorbance of the ADC in PBS buffer at 380 nm, and 280 nm
- Determine the absorbance ratio R:
  $R = A_{380}/A_{280}$
- Estimate DAR by using the formula:
  $DAR = (34.43 \times R)/(3.44 - R)$

Aggregation and Precipitation Issue for Doxorubicin conjugation

This kit is designed to minimize the aggregation and precipitation issues generally occurring with Doxorubicin conjugation. Higher DAR will cause ADC aggregation or precipitation. The recovery is DAR and antibody dependent, and typically around 60%. Aggregation extent can be measured by size-exclusion chromatography (SEC).

Recommended Storage Conditions

mAb-Doxorubicin linakge is cleavable. It is recommended to use the ADC within 24 h. For short time storage, please dilute your ADC in the provided Stabilization Buffer (after diluting from 5x to 1x). Aliquot and store the conjugate at or below -20°C. The storage buffer is biocompatible, with a final composition of 1xPBS and 0.2% Tween, and it can be used directly for any *in vitro* and *in vivo* studies.

In the process, there may be some solid precipitate out during the storage using the stabilization buffer. Please centrifuge or filter before use. Avoid repeated freeze and thaw cycles. The stability of your conjugate may be different due to variation between antibodies and may be checked by SEC HPLC.

Troubleshooting

Low or no conjugation	Buffer containing thiol groups	1. If buffer contains thiol groups, buffer exchange the antibody into a non-thiol containing buffer such as the reaction buffer provided, using protein concentrator or 1x PBS with EDTA by dialysis. 2. Use TCEP reducing agent provided with the kits. If end-users provide their own reducing agent and use DTT, then it needs to be removed prior to the addition of drug-linker.
	Carrier protein was present in the antibody solution	Remove carrier protein before biotinylation by using Protein A, G or A/G resin or an antibody clean-up kit. This will reduce competition for labeling.