Antibody VC-PAB-SN-38 Conjugation Kits

Components

Component		Product size		Storage condition
		BP-50142	BP-50143	
		1 x 1 mg	3 x 1 mg	
A	Mc-VC-PAB-SN38	1 vial	3 vials	-20 °C
B	Reaction Buffer	10 ml	30 ml	4-8 °C
C	DMSO	1	1	Room temp.
D	TCEP	1 x 160 µg	3 x 160 µg	-20 °C
E	Storage Buffer	5X PBS	5X PBS	4-8 °C
F	Protein concentrator	1	3	Room temp.

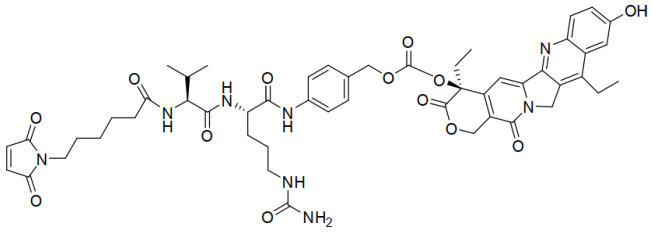
Note:

- The kits are shipped with blue ice.
- When stored as directed, each reagent is stable until the expiration date shown on the bottle label.

Overview

BroadPharm's SN-38 Antibody Conjugation kit attaches the drug, SN-38 to antibody. The kit is designed to utilize the linker's maleimide group to react and attach to thiol groups on the antibody.

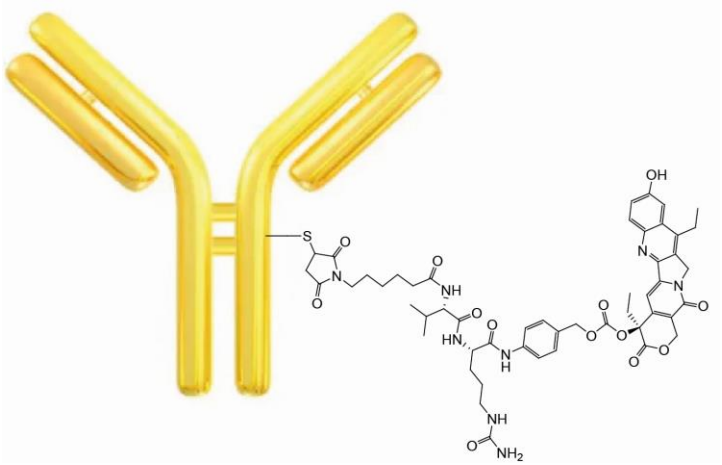
The linker- SN-38 is made by SN38 conjugated to the lysosomally cleavable dipeptide, valine-citrulline (vc) linker. SN-38 is the active metabolite of the topoisomerase-I (topo-I) inhibitor Irinotecan (CPT-11), and it stabilizes the complex between topo-I and DNA which collide with moving DNA replication forks, eventually leading to double stranded DNA damage.



Mc-vc-PAB-SN38
CAS No. : 1801838-28-7
MW:991.07

Kit Features:

- Protocol: easy to follow, to label IgG with SN38 with minimum exposure to the chemotherapeutic drug
- Linkage: releasable linkage
- Fast process: <1 h hands-on time, 6 h 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



ADC

Technical considerations

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

Note

- If you have a different concentration, adjust the antibody concentration accordingly.
- The antibody should be dissolved in reaction buffer or 1X phosphate buffered saline (PBS) pH7.2-7.5 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, mix well, and incubate at 37 °C for 1 hour.
2. Cool the antibody TCEP mixture on ice for 5 minutes.
3. Reconstitute a vial of SN-38 (component A) with 100 µl of DMSO, mix to dissolve.
4. Add all of the cooled antibody mixture from step 3 to the vial of reconstituted SN-38, and mix (final DMSO concentration should be 10% or less). Incubate with rotation, at room temperature for 1 hour.
5. Remove excess SN-38 reagent by protein concentrator by following the instruction for desalt.

De-salt step

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

1. Hydrate concentrator membrane 'filter device' with ~ 500 µl of reaction buffer or DI water, and microcentrifuge 14,000 x g, for 1 minutes. Discard, liquid from filter device and collection tube.
2. Spin down by adding antibody drug conjugate (ADC) to the concentrator/filter device up to 500 µl. Microcentrifuge at 14,000 x g, 8 – 10 minutes, or to minimum volume ~ 50 µl left in the filter device. Discard the waste from the collection tube.
3. Desalt by adding reaction buffer to the filter device up to 500 µl. Microcentrifuge at 14,000 x g, 8 – 10 minutes, or to minimum volume ~ 50 µl left in the filter device. Discard the waste from the collection tube.
4. Repeat step 3, twice.
5. Collect the ADC from filter device into a microcentrifuge tube.
6. Optional for maximum recovery, add a small amount of reaction buffer (volume determined by the user) to the filter device to rinse out residual antibody, microcentrifuge pulse spin, collect antibody/reaction buffer from filter device, add to the microcentrifuge tube from step 5, mix.

Drug-to-Antibody Ratio (DAR) Estimate

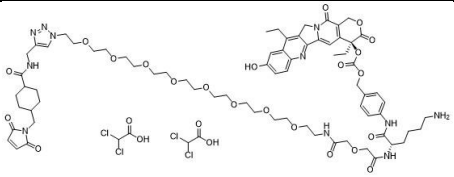
- Obtain absorbance of the ADC in PBS buffer at 380 nm, and 280 nm
- Determine the absorbance ratio R:
  $R = A_{380}/A_{280}$
- Estimate DAR by using the formula:
  $DAR = (34.43 \times R)/(3.44 - R)$

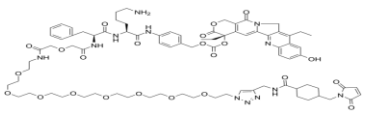
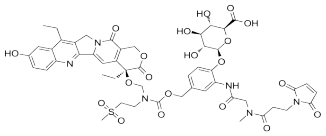
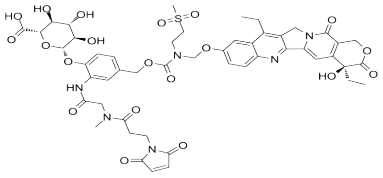
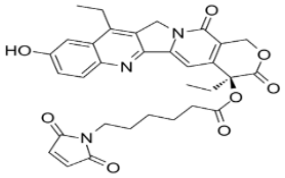
Aggregation and Precipitation Issue for SN38 Labeling

This kit is designed to minimize the aggregation and precipitation issues generally occurring with SN38 labeling. SN-38 is a hydrophobic drug. Higher DAR will cause ADC aggregation or precipitation. The recovery is DAR and antibody dependent, and typically around 60%. Aggregation extent can be determined by size-exclusion chromatography (SEC).

ADC with other linker linked SN-38 conjugation

For other linker linked SN38, such as Cl2A-SN-38, Cl2-SN-38, MAC glucuronide α-hydroxy lactone-linked SN-38, and MAC glucuronide phenol-linked SN-38, please contact us for custom conjugation service.

Name	Structure
Cl2A-SN-38	 CAS No. : 1279680-68-0

Cl2-SN-38	 CAS No. : 1036969-20-6
MAC glucuronide α-hydroxy lactone-linked SN-38	 CAS No. : 2246380-70-9
MAC glucuronide phenol-linked SN-38	 CAS No. : 2246380-69-6
MC-SN38	 1473403-87-0

Recommended Storage Conditions

SN38-Ab linakge is releasable. It is recommended to use ADC within 24 h. For short time storage, please dilute your ADC in Stabilization PBS buffer (5x). Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness.

In the process, there may be some solid precipitate out during the storage using the stabilization buffer. Please centrifuge or filtrate before use. Avoid repeated freeze and thaw cycles. If the ADC is in a lyophilized powder, after dissolving, the solution should be used immediately within 24 h. The stability of your conjugate may be different due to your antibody and should be checked by SEC HPLC.

ADC Stabilizing Buffer

Proprietary ADC stabilizing PBS buffer (5x) contains 5x PBS buffer and other stabilizers to prevent the hydrophobic drugs from interacting with each other during storage and causing the ADCs to precipitate out. Stabilization buffer also helps preserve the structure of the ADCs during lyophilization. The buffer is biocompatible and can be used directly for any in vitro and in vivo studies. For more information on the stabilization buffers, please check our website.

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.