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AqT™ Antibody SN38 Conjugate Evaluation Kit (CM81380 and CM81380.1) User Reference Guide

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. The performance of this kit in labeling may be affected by many different variables, including but not limited to the purity and complexity of the starting materials, differences in preparation techniques, operator ability, and environmental conditions.

Sample data are provided for illustration and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

For Research Use Only. Not for Use in Diagnostic Procedures.

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NOTICE TO CUSTOMER: PROPRIETARY MATERIALS

CellMosaic's AqT™ linkers and products may be covered by one or more domestic and/or international patents and patent applications, including without limitation U.S. Patent Nos. 9,688,663B2; 8,907,079B2; 9,511,150B2; 9,907,854B2; and equivalent patents and patent applications in other countries.

Solely to facilitate the Customer's evaluation of CellMosaic's Proprietary Materials for Customer's internal development and drug screening, CellMosaic has agreed to release limited quantities of its Proprietary Materials (i.e., AqT™ antibody drug conjugate (ADC) and protein drug conjugate (PDC) evaluation kits) to Customer solely for such Customer's practice of the labeling of its antibody or protein using the Proprietary Materials, and to evaluate the benefit such Proprietary Material on the drug performance *in vitro*. The transfer and usage of this kit is governed exclusively by "General Terms and Conditions for Material Transfer of AqueaTether™ (AqT™) Antibody Drug Conjugate (ADC) and Protein Drug Conjugate (PDC) Evaluation Kits", and any supplemental terms set forth in the Quote(s) to which these Terms are attached. Further information can be obtained by contacting:

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Kit Components

The AqueaTether™ (AqT™) antibody SN38 conjugate evaluation kit is one of the few ADC kits currently available for customers to perform conjugations in-house and evaluate the benefit of AqT™ linkers for ADC on a small scale. The kit uses SN38 modified with a super-hydrophilic, water-soluble, and charge neutral AqueaTether™ (AqT™) linker to improve its water solubility and decrease its non-specific interactions with neighboring drugs after conjugation. The end result is an ADC with exceptionally high loading with no or minimum aggregation. The kit is configured for labeling either one 1 mg (Cat#: CM81380.1) or 3 mg antibody sample (Cat#: CM81380).

Table 1: Components and Storage Temperatures for AqT™ Antibody SN38 Conjugate evaluation Kits.

⚠ Upon receipt, please remove **Box 1** and store in a freezer at or below -20°C.
Store **Box 2** in a refrigerator at 2-8°C.

	Name	Part #	Antibody Amount (Kit Cat #)	Quantity
Box 1	AqT™ SN38 Acid (red label) (only one part # will be supplied)	CM81301.3	3 mg (CM81380)	1 unit
		CM81301.1	1 mg (CM81380.1)	
	Reagent A (orange label)	CM80900	Any	1 unit
	Reagent B (green label)	CM80901		1 unit
Box 2	Labeling Buffer (blue label)	CM80902	Any	8 mL
	PBS Buffer (grey label)	CM02013		20 mL
	ADC Stabilizing PBS Buffer (5x) (pink label)	CM02013		0.5 mL
	Desalting Column (only one part # will be supplied)	CM03SG10	3 mg (CM81380)	1
		CM03SG05	1 mg (CM81380.1)	
	Centrifugal Filter Device	CM03CD050A	Any	2
	Collection Tubes	CM03CT0		4
	1.5 mL Centrifuge Tube	CM03CT2		1
	2 mL Centrifuge Tube	CM03CT3		1
	Hazardous Waste Bag	CM03HZ1		1
User Material	IgG Antibody	N/A	NOT PROVIDED (User Supplied Material) **1 mg IgG** for CM81380.1 **3 mg IgG** for CM81380	

Safety Information

Warning: Some of the chemicals used can be potentially hazardous and cause injury or illness. Please read and understand the Safety Data Sheets (SDS) available at CellMosaic.com before you store, handle, or use any of the materials.

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AqT™ Antibody Drug Conjugate

AqT™ linkers are novel proprietary biomaterials invented at CellMosaic that are chemically assembled from a class of natural and edible sugar alcohol compounds with properties by design. AqT™ linkers can be designed to label antibodies with very hydrophobic drugs to improve the overall performance of an ADC as a safer and more effective drug.

SN38 is an inhibitor of topoisomerase I that eventually leads to inhibition of both DNA replication and transcription. On April 7, 2021, the FDA granted regular approval for sacituzumab govitecan (Trodelvy, Immunomedics, Inc.), an ADC that consists of SN-38 linked with a humanized IgG antibody targeted against TROP-2, for the treatment of patients with metastatic triple-negative breast cancer (mTNBC) following at least two prior therapies for metastatic disease. TROP-2 is a cell-surface glycoprotein expressed in more than 90% of TNBCs. Unlike highly toxic chemicals, such as DM1 and MMAE, ADCs with chemotherapeutic drugs may need much higher loading to achieve a therapeutic index.

SN38 is a very hydrophobic molecule. Antibodies labeled with SN38 with classical linkers tend to aggregate and may precipitate out from solution over time. SN38 with AqT™ linker greatly increases the hydrophilicity and water solubility of SN38. The hydroxy (-OH) groups of AqT™ form a network of hydrogen bonds (H-bond) with neighboring water and create a microenvironment that shields neighboring SN38 from stacking or interacting with one another (**Figure 1**). This AqT™ enhanced H-bond network also protects the antibody from enzymatic degradation and retains its activities.

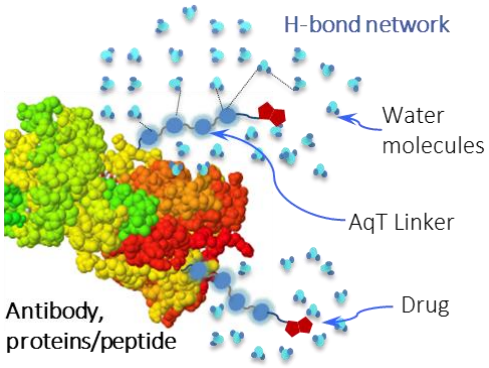

Figure 1. AqT™ enhanced H-bond network

The CM81380 kit is designed to label any antibody (IgG type) with a high amount of SN38 with a minimal amount of added aggregation (see data in **Other Considerations**). The drugs are loaded onto the antibody through an amide bond via the surface amines of the antibody (**Figure 2**). After AqT™ ADC reaches the target, the hydrophilic AqT™ linker can be separated from SN38 via its releasable ester bond. This tactic (traceless release) allows: 1) efficient transportation of the ADC in the biological media with minimum aggregation and toxicity, 2) preservation of the bystander killer effect of SN38.

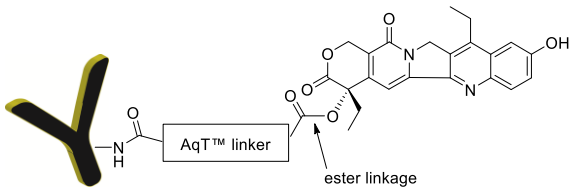

Figure 2. Structure of Antibody-AqT™-SN38

Summary of the key features of the CM81380 evaluation kit:

- Proprietary super-hydrophilic and water-soluble linkage
- High loading with average 5 to 7 SN38 per antibody with minimum added aggregation
- Sugar alcohol-based AqT™ linker preserves the properties of the antibody

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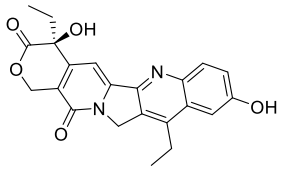
- Releasable linkage with fully released SN38 (traceless release) to maximize the efficacy
- Fast and easy preparation: 4 h preparation and <1 h hands-on time

Table 1: Comparison of ADC products made by the AqT™ Antibody SN38 conjugate evaluation kit (CM81380) and Antibody SN38 Conjugation Kit with Classical Linker (CM11408).

	ADC by CM81380 (AqT™ Ab SN38 Evaluation Kit)	**ADC by CM11408 (Ab SN38 Conjugation Kit with Classical Linker)**
Proprietary	Yes (composition of matter protected by AqT™ molecules)	No, if your antibody is generic
Linker Properties	Proprietary super-hydrophilic and water soluble AqT™ linker	Classic hydrophobic ethylene-type linker
Spacer	Flexibile long spacer (>20 atoms, the exact length is not disclosed here)	Short spacer (4 atoms)
Releasable Chemistry	Same (ester bond)	Same (ester bond)
Loading	High: 5−7 SN38 per antibody	Low: 2−4 SN38 per antibody
Hydrophobicity	Slight changes in hydrophobicity	Dramatically increases hydrophobicity
Aggregation by SEC	No or less than 2% added aggregation	Average 10−50% added aggregation
Heterogenicity by HIC	Less heterogenicity	Highly heterogenous product
Stability	**Stable even with high loading** Can be easily stored frozen without any stabilizer	**Unstable even with low loading**

Drug Information:



- **Name**: SN38 (7-ethyl-10-hydroxycamptothecin)
- **CAS number**: 86639-52-3
- **Chemical formula**: $C_{22}H_{20}N_2O_5$
- **MW**: 392.41
- **Mechanism of action**: Inhibition of topoisomerase I leads to inhibition of both DNA replication and DNA transcription
- **Medical usage**: Pro-drug irinotecan (brand name: Camptosar) is used for treatment of colon and small cell lung cancer

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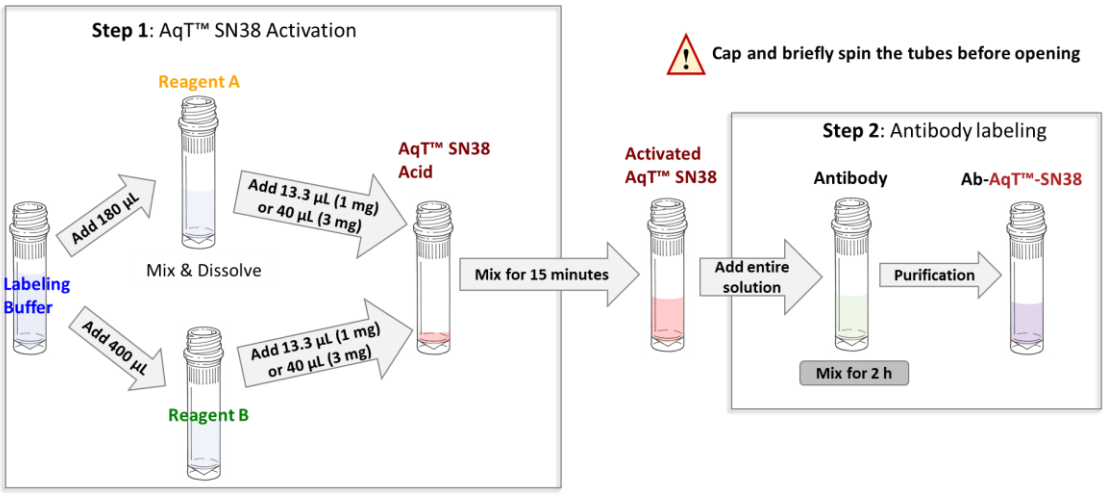
Requirement for antibody (IgG):
1. Preferably > 90% pure by gel electrophoresis
2. Total amount: 1 mg or 3 mg protein content as measured by UV. Note: the accuracy of your protein amount is the single most important factor to obtaining an optimized DAR. Please refer to the section Other Considerations in this manual to measure the protein amount

Support

Customers can request a recommendation for the conjugation if the molecule has a special feature or the amount of antibody is low. CellMosaic also provides additional support services for customers who need help analyzing the final conjugates by HPLC.

Protocol



Scheme 1. Schematic diagram of the workflow for preparing AqT™ antibody-SN38 conjugates.

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 *g* capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C or RT
- Chemical hood
- Support stand, lab frame, or any support rod for desalting column
- Flask
- Personal protection equipment (PPE) (lab coat, safety glasses, and chemical-resistant nitrile gloves)

2. Prepare Site and Reagents for Labeling Experiment

> **Note:** AqT™ SN38 is loaded onto the antibody via a releasable linker. To minimize the release of the payload during storage, it is recommended that the labeling experiment be planned right before your other experiments. If not possible, then please use the ADC stabilization PBS buffer to store under recommended conditions.

Ensure you use PPE (lab coat, safety glasses, and chemical-resistant nitrile gloves) while handling SN38. Locate a clean space inside a chemical hood.

A1. Remove box 1 containing AqT™ SN38 acid (red label), Reagent A (orange label), and Reagent B solution (green label) from the -20°C freezer and warm to RT.
A2. Remove box 2 from the refrigerator. Take the hazardous waste bag and place it inside the chemical hood for solid waste disposal. Bring the rest of the items to a lab bench.
A3. Briefly spin the centrifuge tube containing AqT™ SN38 acid. Place the AqT™ SN38 acid tube in a tube holder inside a chemical hood and wait until the antibody is ready for conjugation.

> **Tip for opening centrifuge tubes after mixing**: Always spin the tubes to ensure no liquid is in the cap.

A4. Set the temperature of the incubator or shaker to 25°C.

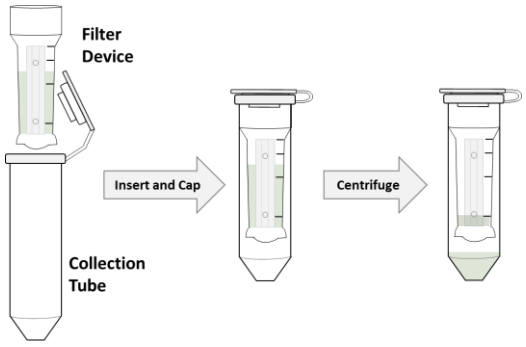
3. Preparation of Antibody Samples for Conjugation

Items needed: Filter Devices (CM03CD050A), Collection Tubes, Labeling Buffer (CM80902, blue label), 1.5 mL Centrifuge Tube, Clean Centrifuge Tubes (not provided in the kit).

Total amount of antibody used for the conjugation is 1 mg or 3 mg (protein content measured by UV) per reaction.

B1. Insert the **Filter Device** into one of the provided collection tubes (microcentrifuge tube with the cap attached). Perform this step based on the following conditions.

- If your antibody is supplied as a lyophilized solid, dissolve the antibody in 500 µL of deionized water and then transfer the entire contents to the Filter Device.
- If your antibody is supplied in < 500 µL buffer, transfer your antibody sample to the Filter Device directly. Add Labeling Buffer to make up the total volume to 500 µL and cap it.
- If the volume of your antibody sample is between 500 and 1000 µL, divide the volume into two Centrifugal Filter



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Devices. Add Labeling Buffer to make up the total volume in each filter device to 500 µL and cap them.

- If the volume of your antibody sample is >1000 µL, add up to 500 µL of sample to each of the two Filter Devices and cap them. Repeat **Steps B1-B4** until all of the antibody sample goes into the Filter Device. Move on to **Step B5**. Add Labeling Buffer to make up the total volume to 500 µL in each device for the last refill.

B2. Place the capped Filter Device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

B3. Spin the Filter Device at 14,000 x *g* for 8 minutes (preferably cooled to 4°C) to concentrate to < 100 µL. (Spin time depends on many factors. The typical spin time for a 500 µL sample is approximately 8 to 20 minutes. The typical volume is ~40 µL after spinning for 8 minutes in an Eppendorf 5417R at 4°C).

B4. Remove the assembled device from the centrifuge and separate the Filter Device from the collection tube. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

B5. Insert the Filter Device back into the collection tube. Add 400 µL of Labeling Buffer to make up the total volume to 500 µL. Place the capped Filter Device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device. Spin the device at 14,000 x *g* to concentrate to < 100 µL. Remove the assembled device from the centrifuge and separate the Filter Device from the collection tube. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

B6. Repeat **Step B5** one time.

B7. Transfer the concentrated sample from the Filter Device to a 1.5 mL micro-centrifuge tube (use the pipetman to estimate the approximate volume of the concentrated sample).

B8. Follow the instructions below for individual kit configurations.

- **1 mg (CM81380.1):** wash the filter two times with **50 µL** of Labeling Buffer and transfer the wash to the tube from **Step B7**. Add Labeling Buffer to make up the total volume of the sample to ~**197 µL** and cap it.
- **3 mg (CM81380):** wash the filter two times with **200 µL** of Labeling Buffer and transfer the wash to the tube from **Step B7**. Add Labeling Buffer to make up the total volume of the sample to ~**590 µL** and cap it.

  Wash = Add buffer, aspirate with pipette 2-3 times.

B9. Vortex the combined antibody sample for 30 seconds and then spin down.

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4. Activation and Antibody Labeling

Items needed: AqT™ SN38 Acid (CM81301.1 for 1 mg scale or CM81301.3 for 3 mg scale, red label), Reagent A (CM80900, orange label), Reagent B (CM80901, green label), Labeling Buffer (CM80902, blue label), Antibody Solution from **Step B9**.

C1. Spin the centrifuge tubes containing Reagent A (orange label) and Reagent B (green label) before opening it.

C2. Transfer **180 µL** of Labeling Buffer to the tube containing Reagent A (orange label). Vortex for 15 seconds to mix and then spin down.

C3. Follow the instructions below for individual kit configurations.

- **1 mg (CM81380.1):** Transfer **13.3 µL** of Reagent A solution from **Step C2** to the tube containing AqT™ SN38 Acid (red label).
- **3 mg (CM81380):** Transfer **40 µL** of Reagent A solution from Step C2 to the tube containing AqT™ SN38 Acid (red label).

C4. Vortex for 15 seconds to mix and then spin down.

C5. Transfer **400 µL** of Labeling Buffer to the tube containing Reagent B (green label). Vortex for 15 seconds to ensure all of the solid is dissolved and then spin down.

C6. Follow the instructions below for individual kit configurations.

- **1 mg (CM81380.1):** Transfer **13.3 µL** of Reagent A solution from **Step C5** to the tube containing AqT™ SN38 Acid (red label).
- **3 mg (CM81380):** Transfer **40 µL** of Reagent A solution from Step C5 to the tube containing AqT™ SN38 Acid (red label).

C7. Vortex for 15 seconds to mix and then spin down. Let the tube mix at RT for 1 h.

Start Time: _____ End Time:_____

C8. Transfer the entire solution from **Step C7** to the antibody solution from **Step B9**. When you add the **SN38 solution**, place the pipette tip inside the antibody solution and then dispense the SN38 slowly while swirling the pipette tip. **Dispose of the pipette tip and SN38 tube in the solid waste bag**.

C9. Cap the centrifuge tube. Mix at 25°C or RT for 2 h.

Start Time: _____ End Time:_____

Tip for mixing: You can use a nutator, shaker, vortex, or incubator shaker for mixing. If you are using end to end nutating, make sure your centrifuge is capped properly. If you don't have any of

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this equipment, you can let the centrifuge tube sit at the bench with manual mixing by pipetting every 20 minutes.

Time-saving tip: While waiting for the reaction to complete, you can move on to **Step D1** and equilibrate the column for purification.

5. Purification of Conjugate

Items needed: Desalting Column (CM03SG05 for 1 mg scale or CM03SG10 for 3 mg scale), PBS Buffer (CM02013, grey label), 2.0 mL Centrifuge Tube, Hazardous Waste Bag, Antibody Solution from **Step C9**.

D1. In a chemical hood, securely attach the **Desalting Column** to a support stand, lab frame, or any support rod. Remove the top and bottom caps from the column and allow the excess liquid to flow through by gravity. Collect the liquid in a flask.
D2. Add 2.5 mL of PBS buffer for 1 mg scale (or 5 mL of PBS buffer for 3 mg scale) and allow the buffer to completely enter the gel bed by gravity flow.
D3. Repeat **Step D2** four times for 1 mg scale (or 5 times for 3 mg scale).
D4. Spin the **AqT™ SN38-labeled antibody solution** from **Step C9** to ensure there is no liquid in the cap before opening it. Add the entire antibody solution to the column. Allow the sample to enter the gel bed completely. **Dispose of the centrifuge tube in the solid waste bag.** (**Note:** this elution buffer does not contain any of your product, you can let it drain into the waste)
D5. Follow the instructions below for individual kit configurations.

- **1 mg (CM81380.1):** Add 250 µL of PBS buffer to the column and allow the liquid to enter the gel bed completely. Place a clean 2.0 mL centrifuge tube under the column. Add 750 µL of PBS buffer to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.
- **3 mg (CM81380):** Add 250 µL of PBS buffer and allow the liquid to enter the gel bed completely. Place a clean 2.0 mL centrifuge tube under the column. Add 1.25 mL of PBS buffer to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

(**Note:** the first elution buffer does not contain any of your product, you can let it drain into the waste)

D6. Label the tube as your product. **Dispose of the Desalting Column in the solid waste bag and seal the bag. Dispose of the waste following regulations appropriate for your area.**
D7. Determine the concentration and the estimated DAR by UV/Vis spectrophotometry (see **Other Considerations**).
D8. If the ADC is not used immediately for experiments, add an equivalent amount of ADC **Stabilizing PBS buffer (5x)** (pink label) to the ADC from **Step D6**. Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness for long-term storage.

Conjugate is Ready for Your Experiment

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- **Specification for your product:** AqT™ SN38-labeled antibodies have an average drug-to-antibody ratio (DAR) of 6. A typical batch contains >99% conjugated product by SEC and is free of any unreacted drug.

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Other Considerations

1. Hydrophilicity of AqT™ SN38 Acid

SN38 is a very hydrophobic molecule. Antibodies labeled with SN38 with classical linkers tend to aggregate and may precipitate out from solution over time. AqT™ labeling of SN38 dramatically increases its hydrophilicity and water solubility.

For example, C18 HPLC analysis shows that AqT™ SN38 acid has a retention time (Rt) of 1.6 minutes less than SN38 acid with classical linker (11.9% less acetonitrile to elute out) (**Figure 3**). C18 HPLC separates molecules by its hydrophobicity and molecular weight. More dramatic differences were observed using hydrophobic interaction chromatography (HIC). HIC separates molecules mainly by hydrophobicity. Molecules first bind to the column with high salt (in this case, $(NH_4)_2SO_4$) in the aqueous buffer. A decreasing salt gradient is used to elute samples. The more hydrophobic a compound, the more decreased salt gradient will need to elute the sample. Column-bound SN38 acid need additional 1M $(NH_4)_2SO_4$ decreases to elute out compared to AqT™ SN38 acid (**Figure 4**).

Figure 3: Overlay of C18 reversed phase HPLC analysis of SN38 acid and AqT™ SN38 acid.

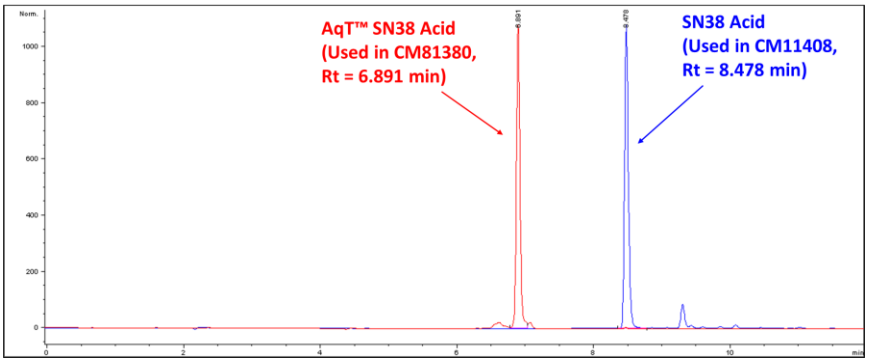
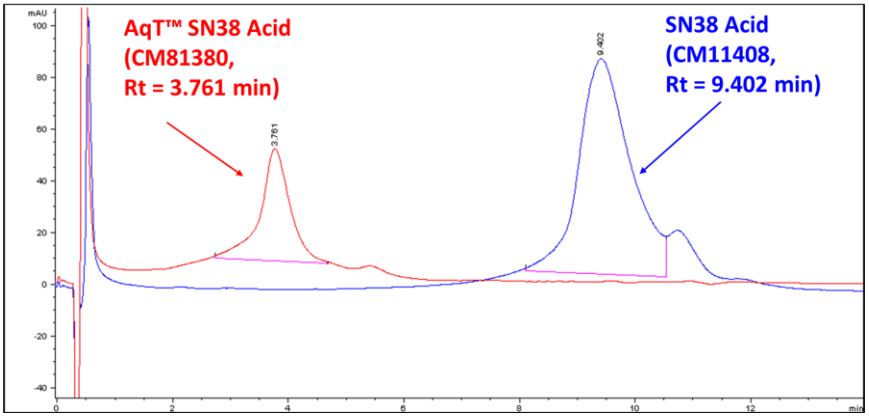


Figure 4: Overlay of HIC HPLC analysis of SN38 acid and AqT™ SN38 acid.



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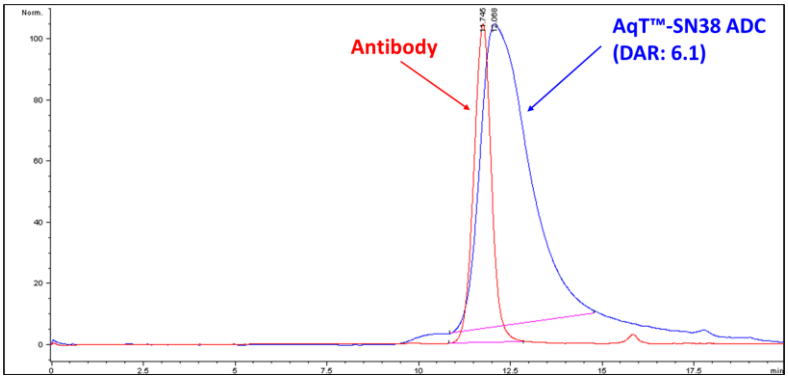
2. Properties of AqT™ SN38 ADC

To determine the benefit of AqT™ linker on the properties of ADCs, size exclusion chromatography (SEC) and HIC were used to analyze AqT™ SN38 ADC produced using CM81380 and compared to the SN38 ADC produced using CM11408.

SEC separates the conjugates by apparent MW or size. The larger MW of the conjugate, the earlier it elutes out. Aggregates usually appear to the left side of the product. For SN38 ADC, there is usually 5 to 50% aggregation for a typical ADC with a loading of 2 to 4. For AqT™ SN38 ADC with a DAR of 6.1, ≤1.2% aggregation was observed (**Figure 5**).

Figure 5: Overlay of SEC HPLC analysis of antibody and AqT™-SN38 ADC (DAR: 6.1).



Due to the highly heterogeneous nature of surface amine labeling, antibody loaded with the same number of drugs (same DAR) may have slightly different hydrophobicity. So, for ADC labeled via surface amine chemistry, a broad peak will be seen in a typical HIC analysis without clear separation of the peaks (**Figure 6**). SN38 ADC is highly hydrophobic. After labeling with an average 4.7 SN38 per antibody, the peak retention time of the antibody increases 1.7 minutes (0.36 minutes per SN38). SN38 antibody is also highly heterogeneous with an HIC peak spanning more than 5.2 minutes (1.1 minutes per SN38). However, for AqT™ SN38 ADC with an average 6.1 AqT™ SN38 per antibody, the increase in hydrophobicity is much smaller (0.1 minutes per AqT™ SN38). AqT™ SN38 labeled antibody is also less heterogeneous with an increase in the peak width of only 0.49 minutes per AqT™ SN38.

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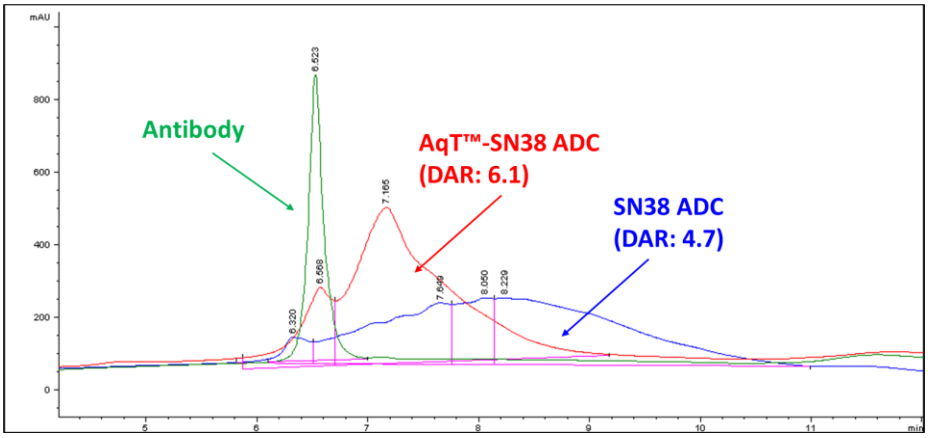
Figure 6: Overlay of the HIC HPLC profiles of antibody, SN38 ADC (DAR: 4.7), and AqT™ SN38 ADC (DAR: 6.1).



3. Concentration Determination for IgG Antibody (Unlabeled)

The accuracy of the IgG amount is important for obtaining an optimized DAR in this protocol. The simplest assay method for determining IgG concentration in solution is to measure the absorbance of the IgG at 280 nm (UV range) ($A_{1\ mg/mL}$ = 1.4).

If your antibody comes with a buffer that has no UV absorbance at 280 nm, you can measure the UV absorbance prior to starting an experiment.

$$Concentration\ (mg/mL)\ of\ IgG = \frac{(A280)}{1.4}$$

If your antibody comes with a buffer that has UV absorbance at 280 nm, you can determine the concentration in **Step B9** after exchanging it with Labeling Buffer and assuming **95%** recovery of the IgG after buffer exchange. Labeling Buffer does not contain any substances that will interfere with UV measurement at 280 nm. The total volume of Labeling Buffer added in **Step B9** can be estimated based on the initially estimated amount of antibody and will not affect the conjugation too much if the volume is a little off.

$$Concentration\ (mg/mL)\ of\ Starting\ IgG = \frac{(A280)}{1.4 \times 0.95}$$

4. Concentration Determination for ADC

To determine the concentration of the ADC, dilute your conjugate from **Step D6** with 1x PBS buffer. Measure the UV absorbance of the conjugate at 280 nm (A280) using a UV spectrometer and calculate the concentration based on the following formulas:

$$Concentration\ (\mu M)\ of\ the\ dilute\ sample = \frac{(A280) \times 1000000}{L\ (210000 + n \times 6100)}$$

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$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280) \times 150000}{L(210000 + n \times 6100)}$$

Where **L** is the UV cell path length (cm). If you are using a 1 cm UV cell, you can dilute the conjugate 4 times to obtain a good reading.

Where **n** is the average molar ratio of SN38 per antibody. Use 6.0 if you do not have the experimental value of your conjugates.

For a typical IgG with MW of 150,000, the molar extinction coefficient is 210,000 $M^{-1}cm^{-1}$. The molar extinction coefficient for SN38 is 6100 $M^{-1}cm^{-1}$ based on CellMosaic's experimental data.

5. MW Calculation

The MW of the conjugate is approximately 160 KDa.

6. Drug-to-Antibody Ratio (DAR) and Characterization by UV and HPLC

In this kit, the target DAR is 6.

The maximum UV absorbance of the antibody (280 nm) and SN38 (380 nm) are shifted to some extent after conjugation with AqT™ SN38. To estimate the DAR, you can obtain the UV absorbance ratio (R) of your conjugate at 366 nm and 270 nm.

$$R = {}^{(A366)}\Big/_{(A270)}$$

The unlabeled antibody will have no absorbance at 366 nm. An AqT™ SN38-ADC with DAR of 5 – 7 will have an R of 0.43 – 0.58.

You can also use the following formula to calculate the estimated DAR (for reference only):

$$DAR = \frac{34.43 \times R}{(3.44 - R)}$$

7. Recommended Storage Conditions

AqT™ SN38 labeled antibody is very stable and can be stored frozen without any stabilizer. However, because AqT™ SN38 is linked to antibody through a releasable linker, we recommend using AqT™ SN38 antibody within a few days if stored at 2-8°C. If you need to store the ADCs for longer term, please dilute your ADC in Stabilization PBS buffer (5x) (included in this kit). Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness. Avoid repeated freeze and thaw cycles.

8. Submit Samples for HPLC Analysis

If you are submitting samples to CellMosaic for SEC analysis, please follow these instructions:

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1) Go online: https://www.cellmosaic.com/hplc-analysis/, select SEC HPLC Analysis (Product# AS0023) and HIC HPLC Analysis (Product#: AS0025), choose the quantity (number of samples. Bulk discounts available for multiple samples) and submit the order. Alternatively, you can email info@cellmosaic.com for a quote and to place the order.
2) Dilute your unconjugated antibody to 1 mg/mL in PBS buffer, then transfer 50 µL of the diluted solution to a 500 µL microcentrifuge tube. Label the vial properly.
3) Transfer 50 µL of ADC (non-diluted solution) to a 500 µL microcentrifuge tube and label the vial properly.
4) Ship your samples with a cold pack for overnight delivery.