

PerKit™ Antibody Deruxtecan High Loading Conjugation Kit (CM11434x1 and CM11434x3) User Reference Guide

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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

Sample data if provided, is provided solely for illustrative purposes and as examples of a small dataset used to verify kit performance within the CellMosaic laboratory. Information regarding the chemicals and reagents used in the kit is included where necessary.

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Kit Components

Trastuzumab Deruxtecan, sold under the brand name Enhertu, typically has an average of 8 molecules of Deruxtecan attached to each antibody molecule. This kit is designed for loading approximately 8 Deruxtecan molecules per antibody. It is only recommended for use after performing conjugation with CM11431 (targeting 4 Deruxtecan per antibody) and confirming that significant aggregations or precipitations was not observed with your antibody using that kit.

The kit provides the materials to conjugate 1 to 3 mg of one antibody sample (CM11434x1) or three antibody samples (CM11434x3) of IgG with Deruxtecan, using enzymatic cleavable peptide linker, GGFG.



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

	Name	Part #	Quantity (CM11434x1)	Quantity (CM11434x3)	Storage condition
Box 1	Deruxtecan Solution (red label)	CM11021.2	0.11 mL	3 x 0.11 mL	-20°C
	Reagent A (blue label)	CM13004	1 unit	3 units	
Box 2	Solution A (green label)	CM01003	1.5 mL	6 mL	2-8°C
	Reducing Buffer (orange label)	CM02086	4 mL	12 mL	
	Labeling Buffer (indigo label)	CM02005	4 mL	12 mL	
	Storage Buffer (1 x PBS Buffer with Stabilizers) (grey label)	CM02022.1	20 mL	60 mL	
	Centrifugal Filter Devices	CM03CD030 A	3	9	
	Desalting Column(s)	CM03SG10	1	3	
	Collection Tubes	CM03CT0	6	18	
	1.5 mL Centrifuge Tubes	CM03CT2	2	6	
	2.0 mL Centrifuge Tube(s)	CM03CT3	1	3	
Hazardous Waste Bag(s)	CM03HZ1	1	3		
User Material	IgG Antibody	N/A	NOT PROVIDED (User Supplied Material, 1-3 mg IgG needed per reaction)		

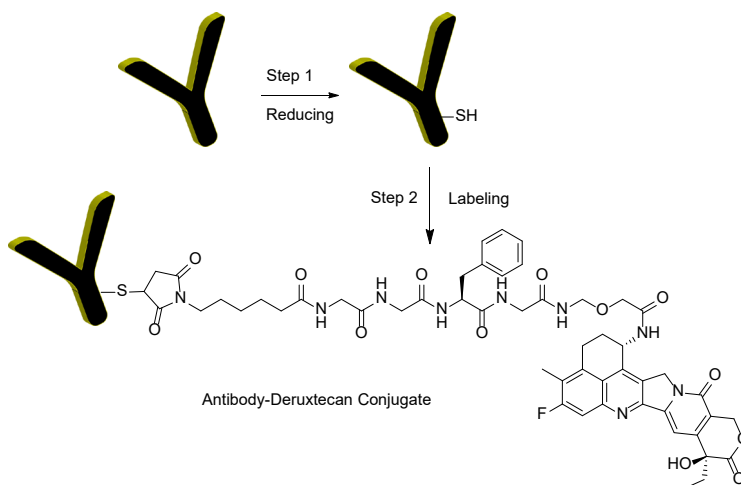
Reaction Scale: The protocol is optimized for conjugating 3 mg of IgG antibody. If you have less than 3 mg of IgG, refer to the calculations in **Steps B10, C3, D9, E2, F5, and F6** to determine the correct volumes to add in each step.

Safety Information

Warning: some of the chemicals used can be potentially hazardous and can cause injury or illness. Please read and understand the Safety Data Sheets (SDS) available at CellMosaic.com before storing, handling, or using any of the materials.

Labeling Chemistry

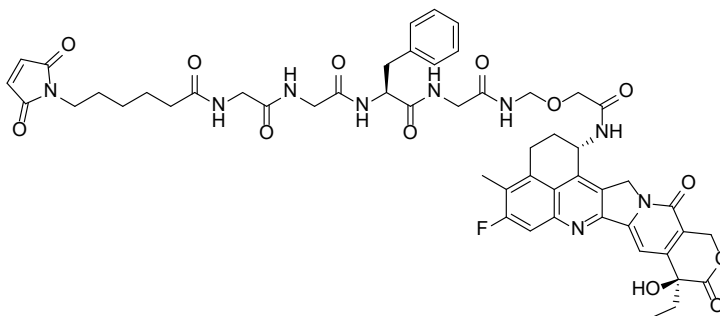
The kit is designed to label any antibody (IgG) with Deruxtecan. Deruxtecan is an exatecan-derivative topoisomerase I inhibitor (DXd) with an enzymatically cleavable peptide linker GGFG (Nakada, T. *et al. Bioorg. Med. Chem. Lett.* **2016**, *26*, 1542–1545). The user supplies the antibody. The kit includes Deruxtecan with a maleimide active group, which can be coupled directly to the antibody after reduction through alkylation in a single step. The final product is purified to remove any unreacted drugs.



Key features of this conjugation kit:

- Offers a simple and easy way to label IgG with Deruxtecan with minimum exposure to the toxin
- Enzymatic cleavable peptide linker GGFG
- High DAR: Up to approximately 8 Deruxtecan molecules per antibody
- Fast and easy preparation: 4 h preparation and <1 h hands-on time
- All reagents and supplies included for preparation and purification
- More than 99% conjugated products (free of any unreacted drugs)

Drug Information:



- **Name:** Deruxtecan (Mc-GGFG-DXd)
- **Synonym:** Exatecan derivative, DX 8951 derivative; DX8951 derivative; DXd; payload for DS-8201a
- **CAS number:** 1599440-13-7
- **Chemical formula:** C₅₂H₅₆FN₉O₁₃
- **MW:** 1034.0684
- **Mechanism of action:** Inhibition of topoisomerase I leads to inhibition of both DNA Replication and DNA transcriptions.
- **Medical usage:** Trastuzumab Deruxtecan, sold under the brand name Enhertu was approved by US FDA for treating metastatic HER2-positive breast cancer.


Requirement for antibody (IgG):

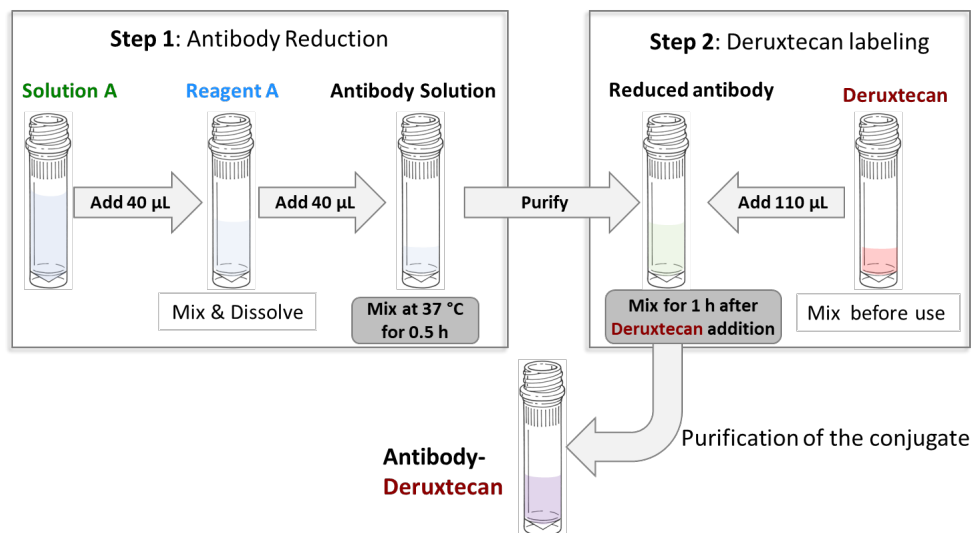
1. Preferably > 90% pure by gel electrophoresis
2. Total amount: 1-3 mg protein content as measured by UV. *Note:* The accuracy of your protein measurement is the single most important factor in obtaining an optimized DAR of 4. Please refer to the "Other Considerations" section in this manual for instructions on measuring the protein amount.

Support

A customer may request recommendations for the conjugation if their IgG has unique features or if they need to label less than 1 mg of IgG. CellMosaic provides additional accessory tools, such as buffers, standards, and reagents for ADC research. We also offer fee-based support services to customers who needing assistance with final conjugate analysis by HPLC and determining the DAR.

Protocol

 Cap and briefly spin the tubes before opening



Scheme 1. Schematic workflow diagram for preparing antibody-Deruxtecan conjugates, starting with 3 mg of IgG. (Reagent volumes will vary if the amount of IgG is less than 3 mg.)

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 g capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker set at 37°C and at RT
- Chemical hood
- Support stand, lab frame, or any support rod for desalting column
- Flask
- Personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves)

2. Prepare Site and Deruxtecan for Labeling Experiment

Deruxtecan is very hydrophobic, and antibody-drug conjugates with Deruxtecan tend to aggregate and precipitate out from solution. Therefore, it is recommended to perform the labeling experiment just a few days before your subsequent experiments.

Always use personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves) when handling Deruxtecan. Ensure you are working in a clean space inside a chemical fume hood.

A1. Remove **Box 1** containing **Deruxtecan** (red label, CM11021.2) and **Reagent A** (blue label, CM13004) from the -20°C freezer and allow it to warm to room temperature before opening the bag.

A2. Remove **Box 2** from the refrigerator. Place the hazardous waste bag inside the chemical hood for solid waste disposal and bring the remaining items to the lab bench.

A3. Check if the frozen liquid inside the **Deruxtecan** tube has thawed. Briefly mix and spin the centrifuge tube containing **Deruxtecan**. Place the **Deruxtecan** 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 briefly to ensure no liquid remains in the cap before opening.

A4. Set the incubator or shaker temperature to 37°C.

3. Preparation of Antibody Samples for Conjugation

Items needed: [Filter Devices \(CM03CD030A\)](#), [Collection Tube \(CM03CT0\)](#), [Reducing Buffer \(CM02086, Dark orange label\)](#), [1.5 mL Centrifuge Tube \(CM03CT2\)](#), [Clean Centrifuge Tubes \(not provided in the kit\)](#).

Total amount of antibody used for the conjugation is 3 mg per reaction (protein content as measured by UV). The protocol is optimized for the IgG antibody with a molecule weight of 150 kDa to obtain an average of 8 drugs per antibody.

Reaction Scale: If you have less than 3 mg of antibody, refer to the calculations in **Steps B10, C3, D9, E2, F5, and F6** to determine the correct volumes to add at each step.

B1. Insert the **Filter Device** into one of the provided collection tubes (micro-centrifuge tube with the cap attached). Follow the appropriate step based on the condition of your antibody.

- ✓ **Lyophilized antibody:** Dissolve the antibody in 500 µL of **deionized water** and transfer the entire contents to the **Filter Device**.
- ✓ **Antibody in < 500 µL buffer:** Transfer the antibody sample directly to the **Filter Device**, then add **Reducing Buffer** to bring the total volume to 500 µL. Cap the device.
- ✓ **Antibody in 500-1000 µL buffer:** Split the sample between two **Centrifugal Filter Devices**, adding the antibody to each device. Add **Reducing Buffer** to bring the volume in each device to 500 µL and cap them.
- ✓ **Antibody in >1000 µL buffer:** Transfer up to 500 µL of the sample into two **Filter Devices**. Cap the devices and repeat **Steps B1-B4** until the entire antibody sample has been transferred. For the final refill (**Step B5**), add **Reducing Buffer** to bring the total volume to 500 µL in each device.

B2. Place the capped **Filter Device** into the centrifuge rotor, ensuring the cap strap is aligned 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 at 4°C) to concentrate the sample to < **100 μL**. (Spin time may vary; typically, a 500 μL sample will concentrate to ~40 μL after 8 to 20 minutes of spinning. The typical time for an Eppendorf 5417R is 8 minutes).

B4. Remove the 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 all experiments are done.

B5. Reinsert the **Filter Device** into the collection tube. Add 400-450 μL of **Reducing Buffer** to bring the total volume to 500 μL. Place the capped **Filter Device** back into the centrifuge rotor, align the cap strap toward the center, and spin at 14,000 x g to concentrate the sample to < **100 μL**. Remove the device, transfer the filtrate to a clean centrifuge tube (not provided). **Save the filtrate until all experiments are done.**

B6. Repeat **Step B5** two more times.

B7. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube. Use a pipetman to measure the approximate volume of the concentrated sample.

B8. Add 20-100 μL of **Reducing Buffer** to the **Filter Device** for rinsing (the actual volume of **Reducing Buffer** will depend on the total volume calculated in **Step B10**). Stir gently with a pipet tip, then transfer the contents to the 1.5 mL micro-centrifuge tube from **Step B7**.

B9. Repeat **Step B8** once.

B10. Add **Reducing Buffer** to the 1.5 mL micro-centrifuge tube from **Step B9** to bring the total sample volume to **260 ± 5 μL** and cap it.

Calculation 1 for Less Antibody (Ab):

$$\text{Total volume of the antibody in Step B10 } (\mu\text{L}) = \text{Ab in mg} \times 86.7$$

B11. Vortex the combined antibody sample for 30 seconds, then briefly spin it down.

4. Antibody Reduction (Step 1 in Scheme 1)

Items needed: Reagent A (CM13004, blue label), Solution A (CM01003, green label), Antibody Solution from **Step B11**, Ice Bath.

C1. Spin the centrifuge tube containing **Reagent A** (blue label).

C2. Spin **Solution A** (green label) briefly before opening. Add **40 μL** of **Solution A** to the tube containing **Reagent A** from **Step C1**. Vortex for 30 seconds to 1 minute to fully dissolve the reagent, then spin briefly.

C3. Add **40 μL** of **Reagent A solution** from **Step C2** to the centrifuge tube containing the antibody from **Step B11**. (Discard of any unused **Reagent A** as hazardous chemical waste **once all experiments are done**).

Calculation 2 for Less Antibody (Ab):

$$\text{Volume of Reagent A solution to be transferred in Step C3 } (\mu\text{L}) = \text{Ab in mg} \times 13.3$$

C4. Vortex the solution for 30 seconds, then spin briefly to ensure no liquid remains in the cap. Incubate the mixture at 37°C for exactly 30 minutes.

Tip for mixing: You can use a nutator, shaker, vortex, or incubator shaker for mixing. If using end-to-end nutating, ensure the tube from **step C4** is securely capped. If you don't have access to this equipment, you can let the tube sit on the bench and manually mixing it by pipetting every 10 minutes.

5. Purification to Remove Excess Reagent A



The following steps should be performed consecutively without interruption, as reduced thiols oxidize quickly. Ensure **step A3** is completed before proceeding. Work quickly through **steps D6-D10**.

Items needed: [Filter Device \(CM03CD030A\)](#), [Collection Tube](#), [Labeling Buffer \(CM02005, indigo label\)](#), [Clean Centrifuge Tubes \(not provided in the kit\)](#), [Antibody Solution from Step C5](#).

D1. Insert the **Filter Device** into one of the provided collection tubes (micro-centrifuge tube with cap attached). Transfer the reduced antibody solution from **Step C4** directly into the **Filter Device**. Rinse the centrifuge tube with 200 μL of **Labeling Buffer** and transfer this solution to the **Filter Device** (total volume 500 μL). Cap the device and place it into the centrifuge rotor, aligning the cap strap toward the center of the rotor. Counterbalance with a similar device.

D2. Spin the **Filter Device** at 14,000 x g for 8 minutes (preferably at 4°C) to concentrate the sample to < 100 μL .

D3. Remove the 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 all experiments are done.

D4. Reinsert the **Filter Device** into the collection tube. Add 400-450 μL of **Labeling Buffer** to bring the total volume to 500 μL . Cap the device and place it back into the centrifuge rotor, aligning the cap strap toward the center of the rotor. Counterbalance with a similar device, and spin at 14,000 x g to concentrate to < 100 μL . Remove the device from the centrifuge and separate the **Filter Device** from the collection tube. Transfer the filtrate to a clean centrifuge tube (not provided). **Save the filtrate until all experiments are done.**

D5. Repeat **Step D4** once.

D6. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube. Use a pipetman to measure the approximate volume of the concentrated sample.

Work quickly

D7. Add 50-200 μL of **Labeling Buffer** to the **Filter Device** for rinsing (the exact volume of **Labeling Buffer** will depend on the total volume calculated in **Step D9**). Stir gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step D6**.

D8. Repeat **Step D7** once.

D9. Add **Labeling Buffer** to the 1.5 mL micro-centrifuge tube to bring the total volume to **640 \pm 10 μL** .

Calculation 3 for Less Antibody (Ab):

$$\text{Volume of Reduced Antibody in Step D9 } (\mu\text{L}) = \text{Ab in mg} \times 213.3$$

D10. Vortex the combined antibody sample for 30 seconds, then briefly spin it down.

6. Deruxtecan Labeling (Step 2 in Scheme 1)

Items needed: Deruxtecan solution from **step A3**, Hazardous Waste Bag (CM03HZ1), Antibody Solution from **step D10**.

E1. While wearing personal protection equipment, carefully open the centrifuge tube containing Deruxtecan from **Step A3**.

E2. Transfer the entire solution (**110 μL** total) to the centrifuge tube containing the antibody from **Step D10**. When adding the Deruxtecan solution, insert the pipette tip into the antibody solution and slowly dispense the Deruxtecan while gently swirling the pipette tip. **Dispose of the pipette tip and Deruxtecan tube in the hazardous waste bag.**

Calculation 4 for Less Antibody (Ab):

$$\text{Volume of Deruxtecan Solution to be Transferred in Step E2 } (\mu\text{L}) = \text{Ab in mg} \times 36.7$$

Dispose of the remainder of the Deruxtecan solution in the hazardous waste bag.

E3. Cap the centrifuge tube and mix at 25°C or room temperature for 1 hour.

Time-saving tip: While waiting for the reaction to complete, you can proceed to **Step F1** and begin equilibrating the column for purification.

7. Purification of Conjugate

Items needed: Desalting Column, Storage Buffer (1xPBS with stabilizers) (CM02022.1, grey label), 2.0 mL Centrifuge Tube (CM03CT3), Hazardous Waste Bag, Antibody Solution from **Step E3**.

F1. 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, allowing the excess liquid to flow through by gravity. Collect the liquid in a flask.

F2. Add 5 mL of **Storage Buffer** to the column and allow it to fully enter the gel bed by gravity flow.

F3. Repeat **Step F2** twice.

F4. Spin the MMAE-labeled antibody solution from **Step E3** before opening the tube. Add the entire antibody solution to the column. **Dispose of the centrifuge tube in the hazardous waste bag.**

F5. Add 250 μL of **Storage Buffer** to the column, allowing the liquid to fully enter the gel bed. (**Note:** This elution buffer does not contain any of your product and can be discarded as waste).

Calculation 5 for Less Antibody (Ab):

$$\text{Volume of Storage buffer in Step F5 } (\mu\text{L}) = 1000 - \text{Ab in mg} \times 250$$

F6. Place a 2 mL centrifuge tube under the column. Add 1.25 mL of **Storage Buffer** to the column and collect the eluent by gravity. Allow the buffer to fully enter the gel bed.

Calculation 6 for Less Antibody (Ab):

$$\text{Volume of Storage buffer in Step F6 } (\mu\text{L}) = 500 + \text{Ab in mg} \times 250$$

F7. Label the tube as your product and store the conjugate at 4°C. **Dispose of the Desalting Column in the hazardous waste bag and seal the bag. Ensure all waste is disposed of in accordance with local regulations.**

Conjugate is Ready for Your Experiment

- **Specifications of your product:** Deruxtecan-labeled antibodies with an average drug-to-antibody ratio (DAR) of approximately 8. A typical batch contains over 99% conjugated products and is free of any unreacted drug. The approximate concentration of the ADC is 1.44 mg/mL in PBS buffer containing stabilizers assuming 60% recovery. You can determine the concentration and estimated DAR of the ADC by MS analysis (see Other Considerations).

Other Considerations

1. Concentration Determination for IgG Antibody (Unlabeled)

Accurately determining the IgG concentration is crucial for obtaining DAR of 4 in this protocol. The simplest method for measuring IgG concentration in solution is to measure the absorbance at 280 nm (UV range), using the formula assuming that 1 mg/mL IgG has an absorbance of 1.4 at 280 nm.

$$\text{Concentration (mg/mL) of IgG} = \frac{(A_{280})}{1.4}$$

If your antibody is in a buffer that does not absorb at 280 nm, you can measure the UV absorbance directly prior to starting an experiment.

If your antibody is in a buffer that absorbs at 280 nm, determine the concentration in **step B10** after buffer exchange with Reducing Buffer, assuming **95%** recovery of the IgG. Reducing Buffer does not interfere with UV measurement at 280 nm.

$$\text{Concentration (mg/mL) of Starting IgG} = \frac{(A_{280})}{1.4 \times 0.95}$$

After calculating the total amount of IgG, follow the calculations in **Steps B10, C3, D9, E2, F5, and F6** to ensure correct volumes are used in each step.

2. Concentration Determination for ADC

To determine the concentration of the ADC, dilute your conjugate from **Step F7** with storage buffer. Measure the UV absorbance of the conjugate at 280 nm (A_{280}) using a UV spectrometer, and calculate the concentration using the following formulas:

$$\text{Concentration } (\mu\text{M}) \text{ of the dilute sample} = \frac{(A_{280}) * 1000000}{L (210000 + n * 6100)}$$

$$\text{Concentration (mg/mL) of the dilute sample} = \frac{(A_{280}) \times 150000}{L(210000 + n * 6100)}$$

Where **L** is the path length of UV cell in centimeters. If you are using a 1 cm UV cell, you can dilute the conjugate 4 times to obtain an accurate reading.

Where **n** is the average molar ratio of Deruxtecan per antibody. Use a value of 8.0 if you do not have the experimental value.

For a typical IgG with a molecule weight (MW) of 150,000, the molar extinction coefficient is 210,000 $\text{M}^{-1}\text{cm}^{-1}$.

3. MW Calculation for ADC

Calculation of the MW of the conjugate:

$$\text{MW(ADC)} = n \times 1104 + 150000$$

Where n is the average molar ratio of Deruxtecan per antibody. Use a value of 8.0 if you do not have the MS data.

4. Characterization of ADC by HIC HPLC

For ADCs prepared via the reduction of antibody thiols, hydrophobic interaction chromatography (HIC) HPLC is used to calculate the DAR and assess the heterogeneity of the ADCs. The conjugates are separated based on hydrophobicity. Antibodies with the same drug-to-antibody ratio (DAR) will have similar hydrophobicity and will elute as a single peak.

For a typical Deruxtecan ADC with an average DAR of 8, a single broad peak is generally observed. Due to the limited information provided by this peak, HIC is not typically useful for calculating the DAR for this high loading. MS spectrum analysis is recommended for more accurate DAR determination.

CellMosaic offers an HIC buffer set ([Product #: CM02025](#)) that can be used with any HIC column. The CM02025 product sheet includes detailed information and methodology for running an HIC HPLC analysis.

If you do not have access to an HPLC facility, you can send your sample to CellMosaic for analysis.

5. Characterization of ADC by SEC HPLC

Deruxtecan is hydrophobic. This kit is designed to minimize the aggregation and precipitation issues typically encountered with deruxtecan labeling. However, you may still notice some solid precipitate or ADC aggregation during the reaction. The precipitate will be removed during purification. Depending on the properties of your antibody, recovery may range from 40-80%.

If you are concerned about aggregation, you can use size exclusion chromatography (SEC) to assess the extent of aggregation. SEC separates conjugates based on apparent molecular weight (MW) or size in aqueous solution. Larger MW conjugate elute earlier. By comparing the SEC profile of unlabeled IgG to that of the ADC, you can estimate the level of aggregation in the ADC.

CellMosaic offers two SEC standards ([Product #: CM92004](#) and [CM92005](#)) for use with any SEC column. The CM92004 product sheet provides all the necessary information and methodology for running an SEC HPLC analysis.

If you do not have access to an HPLC facility, you can send your sample to CellMosaic for analysis.

6. Recommended Storage Conditions

Unlike other ADCs labeled with hydrophobic drugs, ADCs with Deruxtecan are relatively stable even at higher loadings. You may store the ADC solution for a few days at 2-8°C. For long-term

storage, we recommend aliquoting and storing the conjugate in a freezer at temperatures below -20°C , or lyophilizing it to dryness. Avoid repeated freeze-thaw cycles.

7. Sample Submission for HPLC Analysis

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

- 1) Visit CellMosaic's HPLC analysis page (<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 your order. Alternatively, you can email info@cellmosaic.com for a quote and to place the order.
- 2) Dilute your un-conjugated antibody in PBS buffer to a concentration of 1 mg/mL. Transfer 50 μL of the diluted solution into a 500 μL micro-centrifuge tube and label the vial properly.
- 3) Transfer 50 μL of ADC (non-diluted solution) into a 500 μL micro-centrifuge tube and label the vial properly.
- 4) Ship your samples with a cold pack for overnight delivery.

Appendix: Examples of Deruxtecan ADC

Example: Deruxtecan Conjugation with Monoclonal Human IgG1 Subtype Antibody

The ADC was prepared at CellMosaic following the User Manual (Rev. A) without volume adjustments.

Scale of the reaction: 1.0 mg Antibody (Ab)

Specifications of the final conjugates:

Calculated average DAR: 8.0 by HIC

ADC recovery: 61%

Unreacted antibodies: 0%

Aggregation: 0%

Figure 1: HIC HPLC analysis of monoclonal human IgG1 (Panel A), and purified Ab-Deruxtecan conjugates (Panel B).

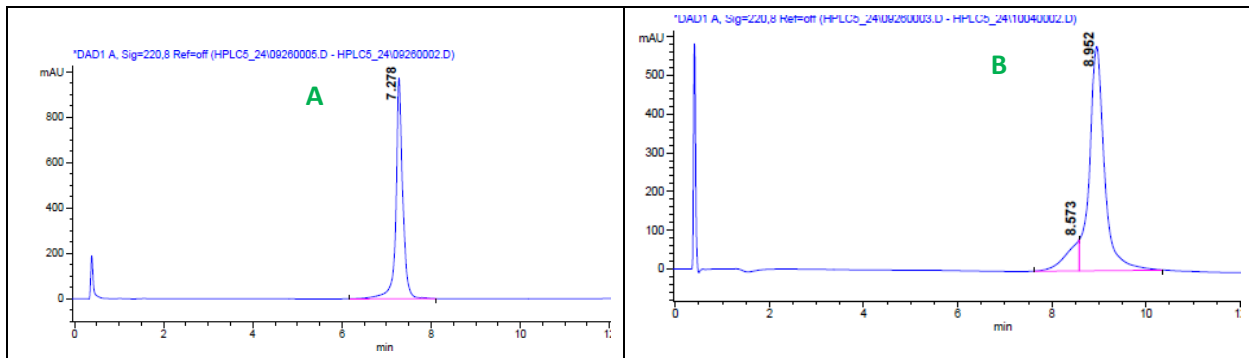


Figure 2: SEC HPLC analysis of monoclonal human IgG1 (Panel C), and purified Ab-Deruxtecan conjugates (Panel D) at 220 and 380 nm. 380 nm absorbance is characteristic of Deruxtecan.

