CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

PerKit™ Antibody MMAF Conjugation Kit (CM11425x1 and CM11425x3) User Reference Guide

Contents

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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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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c/o CellMosaic, Inc.
10-A Roessler Road, Woburn, MA 01801.
Phone： 781-463-0002
Fax： 781-998-4694
E-mail: info@cellmosaic.com

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Kit Components

This kit provides materials to conjugate 1 to 3 mg of a single antibody sample (CM11425x1) or three antibody samples (CM11425x3) (**IgG**) with monomethyl auristatin F (MMAF) using valine-citruline p-aminobenzylcarbamate (VC-PAB) linker.

⚠ 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 #	Quantity (CM11425x1)	Quantity (CM11425x3)	Storage condition
Box 1	MC-VC-PAB-MMAF (**brown label**)	CM11011	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**)	CM02001	4 mL	12 mL	
	Labeling Buffer (**indigo label**)	CM02005	4 mL	12 mL	
	Storage Buffer (1 x PBS buffer) (**grey label**)	CM02013	20 mL	60 mL	
	ADC Stabilizing PBS Buffer (5x) (**pink label**)	CM02022	0.5 mL	1.5 mL	
	Centrifugal Filter Devices	CM03CD050A	3	9	
	Desalting Column	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, use the calculations in **Steps B10**, **C3**, **D9**, **E2**, **F5,** and **F6** to determine the correct volumes to add at each step.

Drug-to-Antibody Ratio (DAR): The protocol is optimized for IgG with a molecular weight of 150 KDa to achieve an average of 4 drugs per antibody (DAR = 4). For other antibodies, the DAR may vary.

Safety Information

Warning: some of the chemicals used in this kit may be 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 these materials.

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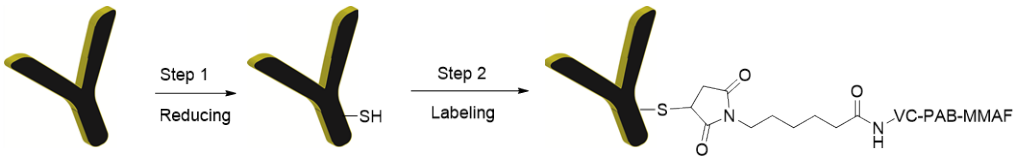
Labeling Chemistry

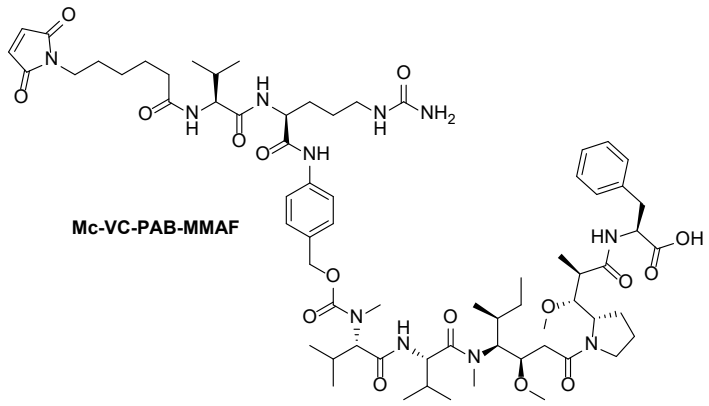
The kit is designed to any IgG antibody labeling of monomethyl auristatin F (MMAF) with a valine-citruline p-aminobenzylcarbamate (VC-PAB) linker (a releasable linkage). The user supplies the antibody. The kit includes maleimide-activated VC-PAB-MMAF, which can be coupled directly to the antibody following reduction and alkylation in a single step (a method developed by Seattle Genetics: Sun *et al*. **2005**, *Bioconjugate Chem*. 16, 1282-1290). The product is then purified to remove any unreacted drugs.



Key features of this conjugation kit:

- Simple and efficient labeling of IgG with MMAF with minimum toxin exposure.
- Features Cathepsin B cleavable VC-PAB linker (Ref. Doronina *et al*., **2008**, *Bioconjugate Chem*. 19, 1960-1963)
- Delivers an average of 4 MMAF molecules per antibody.
- Fast preparation: 4 hours total, with less than 1 hour of hands-on time.
- Includes all necessary reagents and supplies for preparation and purification.
- Achieves over 95% conjugation, free from unreacted MMAF.
- Post-conjugation services available at CellMosaic for analysis and calculation of the DAR.

Drug Information:



- **Name**: Monomethyl auristatin F (MMAF) with Mc-VC-PAB linker
- **CAS number**: N/A
- **Chemical Formula**: $C_{68}H_{103}N_{11}O_{16}$
- **MW**: 1330.63
- **Mechanism of action**: Inhibits cell division by blocking the polymerization of tubulin. VC-PAB linker is stable in extracellular fluid but cleaved by cathepsin B once inside the tumor cell, activating the antimitotic mechanism
- **Activities**: Antioxidant, anti-inflammatory, anticancer, and insecticidal activities

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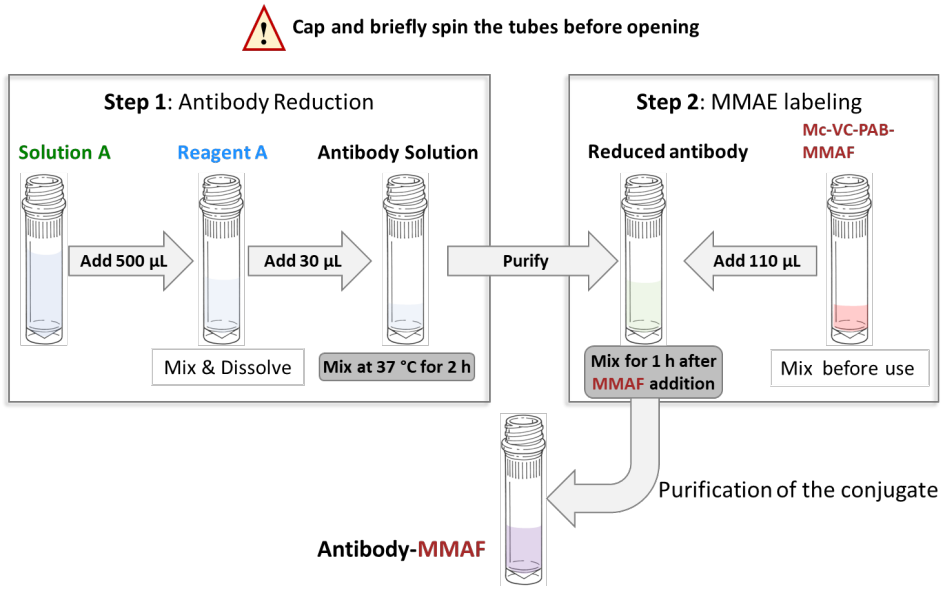
- **Selected references**: 1) Doronina SO, Mendelsohn BA, Bovee TD, *et al*. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. *Bioconjug Chem* **2006**;17: 114–24. 2) Polson AG, *et al*. Antibody-drug conjugates for the treatment of non-Hodgkin's lymphoma: target and linker-drug selection. *Cancer Res.* **2009;**69(6):2358−2364.

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



Scheme 1. Schematic workflow diagram for preparing antibody-MMAF conjugates, starting with 3 mg of IgG. (Reagent volume 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.

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- 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 MMAF for Labeling Experiment

MMAF with VC-PAB is very hydrophobic and antibody-drug conjugates with VC-PAB-MMAF tend to aggregate and precipitate out from the 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 **MMAF** (red label) and **Reagent A** (blue label) 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 **MMAF** tube has thawed. Briefly mix and spin the centrifuge tube containing **MMAF**. Place the **MMAF** 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 (CM03CD050A), Collection Tube (CM03CT0), Reducing Buffer (CM02001, Orange label), 1.5 mL Centrifuge Tube (CM03CT2), Clean Centrifuge Tubes (not provided in the kit).

The 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 4 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.

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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 µ.** (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 **300 ± 5 µL.** Cap the tube.

Calculation 1 for Less Antibody (Ab):

$$Total\ volume\ of\ the\ antibody\ in\ Step\ \boldsymbol{B10}\ (\mu L) = Ab\ in\ mg\ \times 100$$

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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 500 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 **30 µL** of **Reagent A solution** from **Step C2** to the centrifuge tube containing the antibody from **Step B11.** (Dispose of any unused **Reagent A** as hazardous chemical waste **once all experiments are done**).

Calculation 2 for Less Antibody (Ab):

$$Volume\ of\ Reagent\ A\ solution\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Ab\ in\ mg\ \times 10$$

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 (CM03CD050A), Collection Tube (CM03CT0), 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.

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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 ± 10 µL**.

Calculation 3 for Less Antibody (Ab):

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

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

6. MMAF Labeling (Step 2 in Scheme 1)

Items needed: MMAF 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 MMAF from **Step A3**.

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

Calculation 4 for Less Antibody (Ab):

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

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

E3. Cap the centrifuge tube and mix the solution 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.

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Woburn, MA 01801
USA

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7. Purification of Conjugate

Items needed: Desalting Column, Storage Buffer (1x PBS), 2.0 mL Centrifuge Tube (CM03CT3), Hazardous Waste Bag (CM03HZ1), 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 MMAF-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):

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

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):

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

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.**

F8. Determine the concentration and estimate the DAR by UV/Vis spectrophotometry (see "Other Considerations").
F9. If the ADC is not used for the experiment within a few days, add **Stabilization PBS buffer (5x)** (pink label) to the ADC from **Step F7**. Aliquot and store the conjugate in a freezer at temperatures below -20°C, or lyophilize to dryness for long-term storage.

Calculation 7 for ADC Stabilizing Buffer:

$$Volume\ of\ ADC\ Stabilizing\ Buffer\ in\ Step\ \boldsymbol{F9} = Total\ Vol.\ of\ ADC\ \times 0.25$$

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10A Roessler Road
Woburn, MA 01801
USA

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Conjugate is Ready for Your Experiment

- **Specifications of your product:** MMAF-labeled antibodies with an average drug-to-antibody ratio (DAR) of approximately 4. A typical batch contains over 95% conjugated products and is free of any unreacted drug. The approximate concentration of the ADC is 1.44 mg/mL in PBS buffer assuming a 60% recovery. You can determine the concentration and estimate the DAR of the ADC by UV/vis spectrophotometry (see “Other Considerations”).

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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.

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

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.

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

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 1x PBS buffer. Measure the UV absorbance of the conjugate at 280 nm (A280) using a UV spectrometer, and calculate the concentration using the following formulas:

$$Concentration\ (\mu M)\ of\ the\ dilute\ sample = \frac{(A280) \times 4.7619}{L}$$

$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280) \times 0.7143}{L}$$

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

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

3. MW Calculation for ADC

Calculation of the MW of the conjugate:

MW(ADC) = n x 1332+ 150000

Where *n* is the average molar ratio of MMAF per antibody. Use a value of 4.0 if you do not have the experimental data.

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

In this kit, the target DAR is 4. Depending on your antibody, you may achieve a slightly higher or lower DAR.

To estimate the DAR, you can calculate the UV absorbance ratio (*R*) of your conjugate at 248 nm and 280 nm using the following formula.

$$R = {(A248)}/{(A280)}$$

Unlabeled antibody typically has an *R* value between 0.4 and 0.5.

MMAE-ADC with a DAR of 3 to 5 have an *R* value between 0.65 – 0.80.

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

$$DAR = \frac{(21 \times R - 9)}{(1.615 - 0.1425 \times R)}$$

Note: The UV contribution of the VC-PAB-MMAF to the ADC is experimentally determined at CellMosaic. The UV absorbance of the VC-PAB-MMAF in an ADC can vary significantly due to factors like aggregation and stacking. Therefore, the ***R*** value for an ADC may differ greatly depending on the antibodies and should be determined experimentally. The DAR calculation using this formula is for reference purpose only.

5. 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 Mc-VC-PAB-MMAF ADC, multiple peaks indicate different levels of drug-loading.

An example of HIC HPLC profiles for Mc-VC-PAB-MMAF ADCs can be found in the Appendix.

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.

6. Characterization of ADC by SEC HPLC

Mc-MMAF with VC-PAB is highly hydrophobic. This kit is designed to minimize the aggregation and precipitation issues typically encountered with Mc-VC-PAB-MMAF 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

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(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.

7. ADC Stabilizing Buffer

CellMosaic's proprietary ADC Stabilizing PBS buffer (5x) (Product #: CM02022) contains 5x PBS buffer and other stabilizers designed to prevent hydrophobic drugs from interacting with each other during storage, which can lead to ADCs precipitation. The Stabilizing Buffer also helps preserve the structure of the ADCs during lyophilization. This biocompatible buffer can be used directly for both *in vitro* and *in vivo* studies. For more information on stabilization buffers, please visit our website.

8. Recommended Storage Conditions

Recommend storing at 2-8°C for short-term. Do not freeze in PBS buffer. Based on our preliminary data, the conjugate made with this kit remains stable in PBS buffer at 2-8 °C for several weeks. However, you may notice an increased amount of aggregation after some time. The stability of your conjugate may vary depending on your antibody and should be checked either by HPLC or UV.

For long-term storage, dilute your ADC in Stabilization PBS Buffer (5x) (included in this kit). Aliquot and store the conjugate in a freezer at temperatures below -20°C or lyophilize to dryness. Avoid repeated freeze-thaw cycles. If the ADC is in lyophilized powder form, once dissolved, the solution should be used within a few days.

9. 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.

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Appendix: Typical Kit Performance Data (LC analysis, CellMosaic)

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

Antibody information: A therapeutic antibody (Human IgG1 subtype)

Kit Lot number: 5524.S13.021119

Scale of the reaction: 1 mg of antibody (protein content)

Figure 1: SEC HPLC analysis of purified MMAF-ADC with VC-PAB linker.

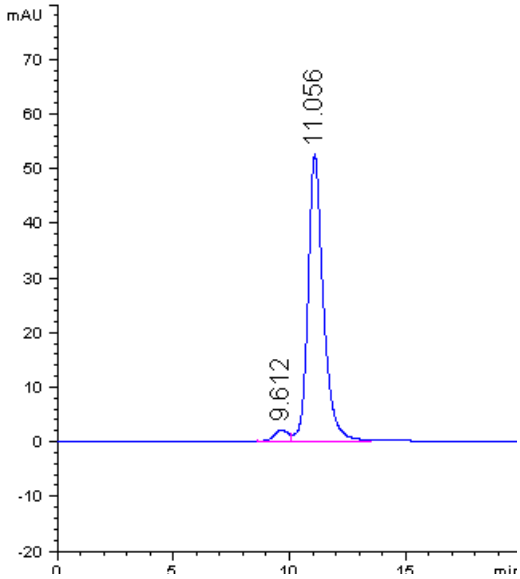
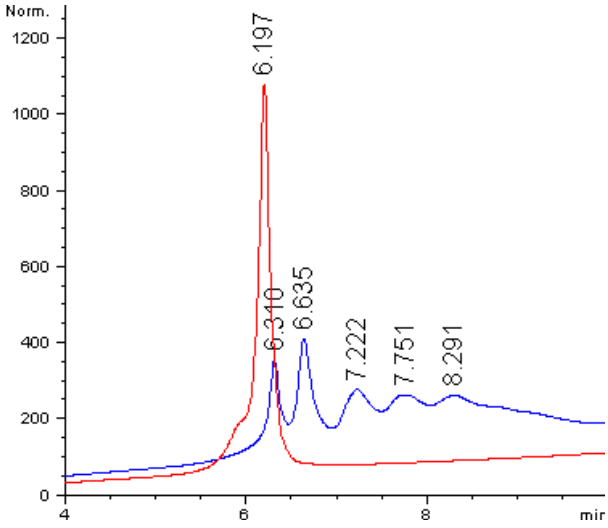


Figure 2: HIC HPLC analysis of antibody (red trace) and purified conjugates (blue trace)



Summary of the results:

R value (consider the total peaks) (SEC)	0.667
Average DAR based on R value	3.3
Average DAR based on HIC HPLC	3.4
Extent of antibody aggregation (%)	3.4
Unreacted antibody (%)	0
Unreacted MMAF (%)	0
Recovery (%)	90