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 MMAE Conjugation Kit (CM11409.01x1 and CM11409.01x3) 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

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.

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

The information in this document is subject to change without notice. CellMosaic assumes no responsibility for any errors or omissions that may appear in this document. Under no circumstances shall CellMosaic be liable, whether in contract, tort, warranty, or under any statute or on other legal theory, for special, incidental, indirect, punitive, multiple, or consequential damages related to or arising from this document, including but not limited to the use thereof.

NOTICE TO PURCHASER: LIMITED LICENSE

Purchasing this product grants a limited, non-transferable license to use it solely for the purchaser's research activities to practice the labeling methods using the reagents. The license granted herein is personal to the original purchaser and may not be transferred to any other party outside the purchaser's company. No other right or license is conveyed or granted either expressly, by implication or by estoppel, to resell or repackage this product. Further information can be obtained by contacting:

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

CellMosaic™

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

Kit Components

This kit provides materials to conjugate 0.1 mg (100 microgram) of a single antibody sample (CM11409.01x1) or three antibody samples (CM11409.01x3) (**IgG**) with monomethyl auristatin E (MMAE) using valine-citruline p-aminobenzylcarbamate (VC-PAB) linker. 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.

⚠ 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 (CM11409.01x1)	Quantity (CM11409.01x3)	Storage condition
Box 1	MC-VC-PAB-MMAE (**red label**)	CM11001.01	5 µL	3 x 5 µL	-20°C
	Reagent A (**blue label**)	CM13004	1 unit	3 units	
Box 2	Solution A (**green label**)	CM01003	2 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	5 mL	20 mL	
	Centrifugal Filter Devices	CM03CD050 A	3	9	
	Collection Tubes for Filter	CM03CT0	6	18	
	Desalting Spin Column	CM03SG50	2	6	
	Collection Tubes for Spin Column	CM03CT9	2	6	
	0.5 mL Eppendorf Tubes	CM03CT7	2	6	
	1.5 mL Centrifuge Tube	CM03CT2	2	6	
	Hazardous Waste Bag(s)	CM03HZ1	1	3	
User Material	IgG Antibody	N/A	NOT PROVIDED (User Supplied Material, **0.1 mg IgG** needed per reaction)		

Safety Information

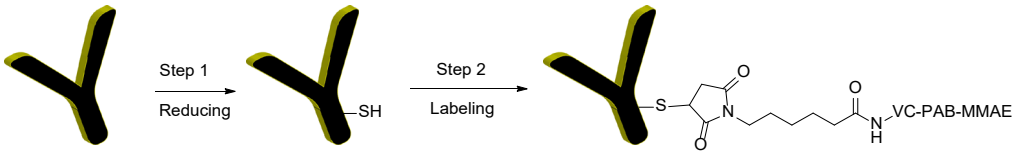
Warning: some of the chemicals used in this kit may be hazardous and can cause injury or illness. Please read and understand the Safety Data Sheets (SDS) available at CellMosaic.com before storing, handling, or using any of these materials.

Labeling Chemistry

The kit is designed to label any IgG antibody with monomethyl auristatin E (MMAE) using a valine-citruline p-aminobenzylcarbamate (VC-PAB) linker. The user supplies the antibody. The kit includes maleimide-activated VC-PAB-MMAE, 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.

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

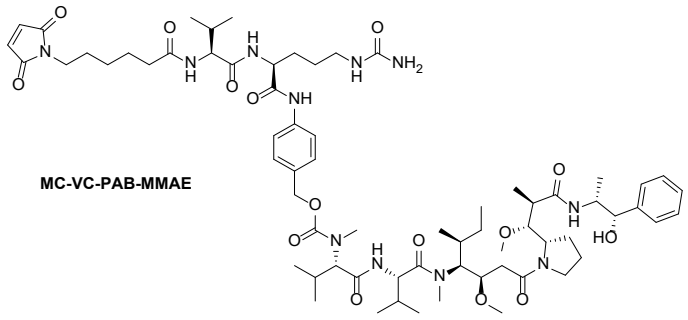
Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com



Key features of this conjugation kit:

- Simple and efficient labeling of IgG1 with MMAE, minimizing toxin exposure.
- Features Cathepsin B-cleavable VC-PAB linker (Ref. Doronina et al., 2008, Bioconjugate Chem., 19, 1960-1963).
- Delivers an average of 4 MMAE 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 MMAE.
- Post-conjugation services available at CellMosaic® for analysis and DAR determination.

Drug Information:



- **Name**: Monomethyl auristatin E (MMAE) with Mal-VC-PAB linkage
- **CAS number**: 646502-53-6
- **Chemical Formula**: $C_{68}H_{105}N_{11}O_{15}$
- **MW**: 1316.65
- **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

Requirement for antibody (IgG1 subtype):
1. Preferably > 90% pure by gel electrophoresis.
2. Total amount: 0.1 mg (100 microgram) 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.

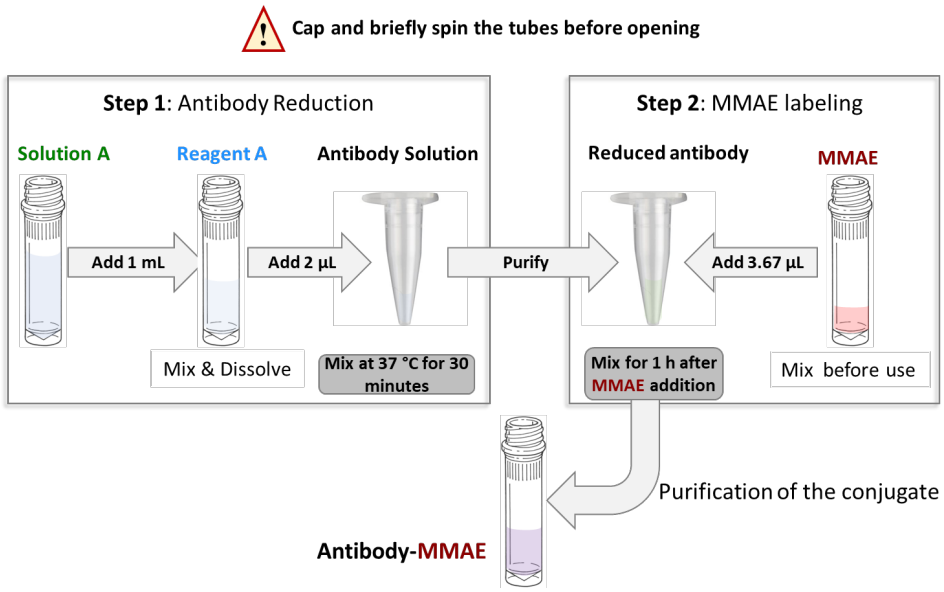
CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

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

Support

A customer may request recommendations for the conjugation if their IgG has unique features or if they need to label less than 100 microgram 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-MMAE 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 37°C or 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 MMAE for Labeling Experiment

MMAE with VC-PAB is highly hydrophobic and antibody-drug conjugates with VC-PAB-MMAE 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.

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

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 **MMAE** (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 **MMAE** tube has thawed. Briefly mix and spin the centrifuge tube containing **MMAE**. Place the **MMAE** tube in a tube holder inside the 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), 0.5 mL Eppendorf Tube (CM03CT7), Clean Centrifuge Tubes (not provided in the kit).

The total amount of antibody used for the conjugation is 0.1 mg (100 microgram) per reaction (protein content as measured by UV).

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.

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

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. For the last repeat, if you start with two **Filter Devices**, combine the samples into one **Filter Device** and spin at 14,000 x g to concentrate the solution to less than **20 µL**.

B7. Transfer the concentrated sample from the **Filter Device** to a 0.5 mL Eppendorf tube. Use a pipetman to estimate the approximate volume of the concentrated sample. Calculate the volume of **Reducing Buffer** needed for rinsing the **Filter Device** in **Step B8**. After combining the concentrated sample from **Step B7** and the rinsing solution from **Step B8**, the total volume should be approximately **30 µL**

B8. Add 10-20 µL of **Reducing Buffer** to the **Filter Device** to rinse. Gently stir the buffer with a pipet tip, then transfer the entire contents to the 0.5 mL Eppendorf tube from **Step B7.**

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

4. Antibody Reduction (Step 1 in Scheme 1)

Items needed: Reagent A (CM13004, blue label), Solution A (CM01003, green label), Antibody Solution from **Step B9**, Ice Bath.

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

C2. Spin **Solution A** (green label) briefly before opening. Add 1 mL 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 **2 µL** of **Reagent A solution** from **Step C2** to the centrifuge tube containing the antibody from **Step B9.** (Discard of any unused **Reagent A** as hazardous chemical waste **once all experiments are done**)

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-D8**.

Items needed: Filter Device (CM03CD050A), Collection Tube (CM03CT0), Labeling Buffer (CM02005, indigo label), 0.5 mL Eppendorf Tube (CM03CT7), 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. Spin the **Filter Device** at 14,000 x g to concentrate the solution to less than **20 µL**.

Work quickly

D6. Transfer the concentrated sample from the **Filter Device** to a 0.5 mL Eppendorf tube. Use a pipetman to estimate the approximate volume of the concentrated sample. Calculate the volume of **Labeling Buffer** needed for rinsing the **Filter Device** in **Step D7**. After combining the concentrated sample from **Step D6** and the rinsing solution from **Step D7,** the total volume should be approximately **30 µL**

D7. Add 10-20 µL of **Labeling Buffer** to the **Filter Device** to rinse. Gently stir with a pipet tip, then transfer the entire contents to the 0.5 mL Eppendorf tube from **Step D6.**

D8. Vortex the combined antibody sample for 30 seconds, then centrifuge to ensure no liquid remains in the cap.

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

6. MMAE Labeling (Step 2 in Scheme 1)

Items needed: MMAE solution from **step A3**, Hazardous Waste Bag (CM03HZ1), Antibody Solution from **step D8**.

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

E2. Transfer **3.67 µL** of MMAE solution from **Step E1** to the centrifuge tube containing the antibody from **Step D8**. When adding the MMAE solution, insert the pipette tip into the antibody solution and slowly dispense the MMAE while gently swirling the pipette tip. **Dispose of the pipette tip and MMAE tube 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.

7. Purification of Conjugate

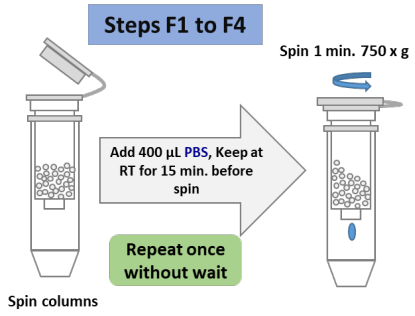
Items needed: Desalting Spin Column (CM03SG50), Storage Buffer (1x PBS) (CM02013, grey label), Collection Tubes for Spin Column (CM03CT9), 1.5 mL Centrifuge Tube (CM03CT2), Hazardous Waste Bag (CM03HZ1), MMAE–Antibody Solution from **Step E3**.

F1. Take out two desalting spin columns and remove the bottom red cap. Spin the columns for 1 minute at 750 x *g* before opening the top cap.

F2. Apply 400 µL of PBS buffer (grey label) to the top-center of the resin in each column. Allow the resin to swell at room temperature for 15 minutes.

F3. Spin the columns for 1 minute at 750 x *g* and discard the flow-through.

F4. Repeat **Steps F2–F3** once. Spin immediately after applying PBS, without wait, and discard the flow-through.

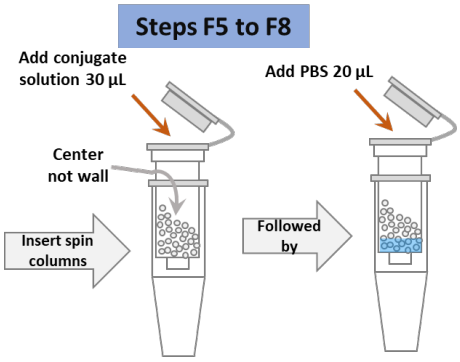


F5. Insert the spin columns into clean 1.5 mL collection tubes.

F6. Spin the MMAE–antibody solution from **Step E3** to ensure no liquid remains in the cap before opening. Add 1xPBS buffer to bring the total volume of the MMAE solution to 60 µL.

F7. Slowly apply up to 30 µL of the conjugate solution from **Step E3** to the top-center of the resin in each spin column, taking care not to disturb the resin bed (2 x 30 µL).

F8. Washing the tube with 40 µL of PBS buffer, then apply 20 µL of PBS buffer to the top-center of the resin in each spin column, bringing the total volume in each



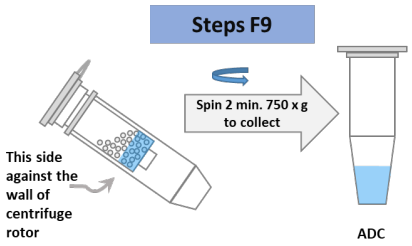
CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

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

column to 50 µL. **Dispose of the centrifuge tube in the solid waste bag.**

> ⚠ The resin may slightly detach from the column to form, forming a pillar with an unbalanced resin bed due to centrifuge force. To prevent issues, ensure that both the sample and subsequent PBS buffer are applied slowly to the center of the resin bed, avoiding any runoff down the sides. Wait for the conjugate solution to fully enter the resin before applying the PBS buffer. Be careful not to touch the resin bed with the pipette tip.

F9. Rotate and align the spin column so that the higher side of the resin bed is positioned against the outer wall of the centrifuge rotor, while lower side faces the center. Spin for 2 min at 750 x *g* to collect the fractions.



F10. Transfer and combine the fractions from the two collection tubes into the provided 1.5 mL centrifuge tube and cap it. **Dispose of the Desalting Spin Columns and Collection Tubes in the solid waste bag, then seal the bag. Follow local regulations for proper waste disposal.**

Conjugate is Ready for Your Experiment

- **Specification for your product:** MMAE-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 0.5 mg/mL in PBS buffer assuming a 50% recovery. You can determine the concentration and estimate the DAR of the ADC by UV/vis spectrophotometry (see other considerations).

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

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 B9** after buffer exchange with Reducing Buffer, assuming **95%** recovery of the IgG. Reducing Buffer does not interfere with UV measurement at 280 nm.

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

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

Calculation of the MW of the conjugate:

MW(ADC) = n x 1317+ 150000

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

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

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-MMAE to the ADC is experimentally determined at CellMosaic. The UV absorbance of the VC-PAB-MMAE 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 MMAE ADC, multiple peaks indicate different levels of drug-loading.

Examples of HIC HPLC profiles for MMAE ADCs with various antibodies 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

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

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

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.

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

Unlike other ADCs labeled with hydrophobic drugs, ADCs with MMAE are relatively stable. Based on our preliminary data, the conjugates made with this kit can remain stable in PBS buffer for several weeks at 2-8°C. Freezing is not recommended.

The stability of your conjugate may vary depending on your specific antibody and should be checked by either HPLC or UV analysis. If you need to store ADCs for an extended period, you can purchase the ADC stabilization PBS buffer separately. Dilute your ADC in Stabilization PBS Buffer (5x), aliquot the conjugate, and store it in a < -20°C freezer, or lyophilize to dryness. Avoid repeated freeze-thaw cycles.

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 CM11409 User Manual (Rev. H) without any adjustments

Antibody information: A therapeutic antibody (Human IgG1 subtype)

Kit Lot number: 5508.S9.020918

Figure 1: HIC HPLC analysis of antibody, Mal-VC-PAB-MMAE, and purified conjugates

Scale of the reaction: 3 mg (CM11409)

Specification of the final conjugates:

Calculated average DAR: 4.86 Percentage of unreacted antibodies: 2.6%;

Percentage of unreacted MMAE: 0% ADC recovery: 81%