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PerKit™ Protein MMAE Conjugation Kit (CM11413 and CM11413x3) 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.

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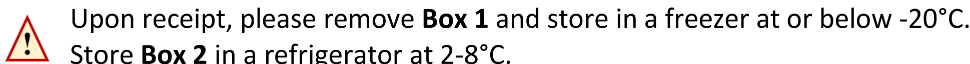
Kit Components

This kit provides materials to conjugate 6.67 to 20 nmole of one (CM11413) or three (CM11413x3) protein samples (≥20 KDa) with monomethyl auristatin E (MMAE) 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 (CM11413)	Quantity (CM11413x3)	Storage condition
Box 1	MC-VC-PAB-MMAE (**red label**)	CM11001	0.11 mL	3 x 0.11 mL	-20°C, dry
	Reagent A (**cyan label**)	CM12101	1 unit	3 units	
	Reagent B solution (**yellow label**)	CM12004.1	1 unit	3 units	
Box 2	Solution A (**green label**)	CM01003	1 mL	3 mL	2-8°C
	Buffer A (**orange label**)	CM02001	4 mL	12 mL	
	Buffer B (**indigo label**)	CM02005	12 mL	36 mL	
	Storage Buffer (1 x PBS buffer) (**grey label**)	CM02013	20 mL	60 mL	
	Stabilization PBS buffer (5x) (**Pink label**)	CM02022	0.5 mL	1.5 mL	
	Centrifugal Filter Devices	CM03CD010A	2	6	
	Desalting Column 1	CM03SG05	1	3	
	Desalting Column 2	CM03SG10	1	3	
	Collection Tubes	N/A	4	12	
	1.5 mL Centrifuge Tubes	N/A	2	6	
	2.0 mL Centrifuge Tube(s)	N/A	1	3	
	Hazardous Waste Bag(s)	N/A	1	3	
User Material	Protein (MW: ≥20 KDa)	N/A	NOT PROVIDED (User Supplied Material, **6.67-20 nmole protein** needed per reaction)		

Reaction Scale: The protocol is optimized for conjugating 20 nmole of protein. If you have less than 20 nmole of protein, use the calculations in **Steps B10**, **C3**, **D5**, **D6, D7, D10, E5,** and **E6** to obtain the correct volumes to be added 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 you store, handle, or use any of the materials.

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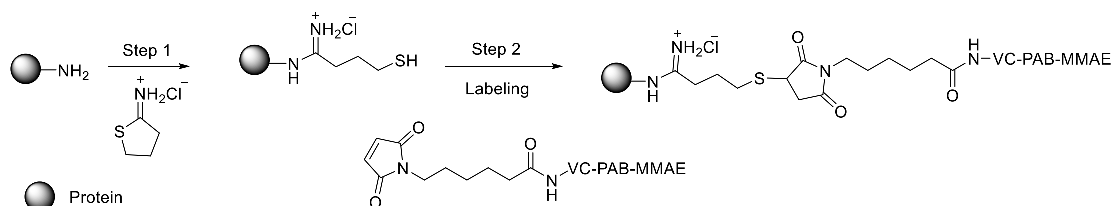
Labeling Chemistry

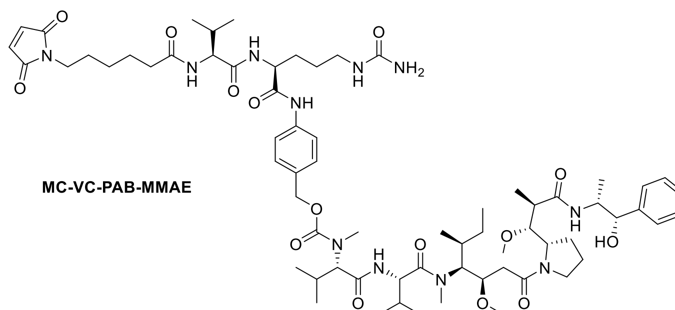
The kit is designed to label any protein with monomethyl auristatin E (MMAE) using a valine-citruline p-aminobenzylcarbamate (VC-PAB) linker. The user supplies the protein. Using the kit components, the user converts the protein to a thiol-protein via surface amine labeling, followed by reaction of the thiol-protein with maleimide-activated VC-PAB-MMAE to generate protein-MMAE conjugates. The product is purified to remove any unreacted drugs.



Key features of this conjugation kit:

- Offers a simple and easy way to label protein with MMAE with minimum exposure to the toxin
- Cathepsin B cleavable VC-PAB linkage (Ref. Doronina *et al*. **2008**, *Bioconjugate Chem*. 19, 1960-1963)
- Fast and easy preparation: 6 h preparation and <2 h hands-on time
- All reagents and supplies included for preparation and purification

Drug Information:



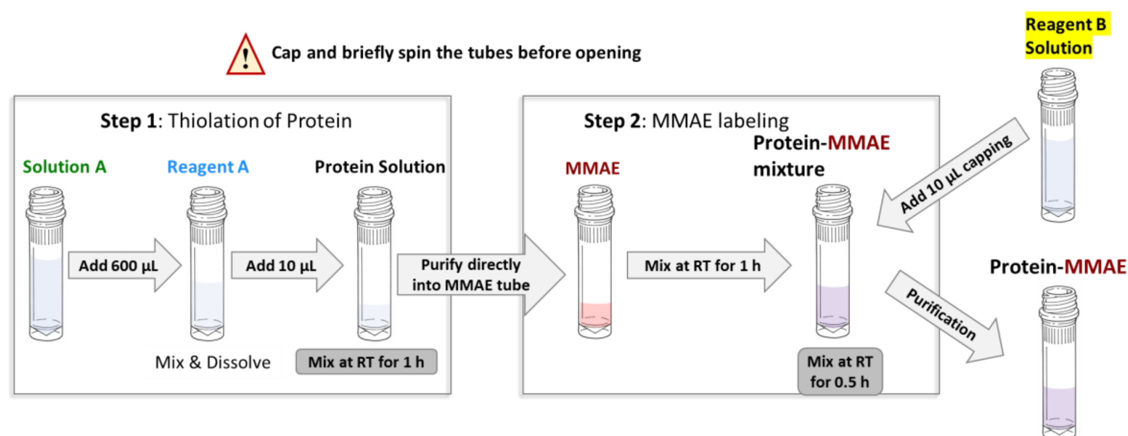
- **Name**: Monomethyl auristatin E (MMAE) with MC-VC-PAB linkage
- **CAS number**: 646502-53-6
- **Chemical formula**: $C_{68}H_{105}N_{11}O_{15}$
- **MW**: 1316.65
  Mechanism of action: Inhibits cell division by blocking the polymerization of tubulin, the VC-PAB linker is stable in extracellular fluid but cleaved by cathepsin B once inside the tumor cell, activating the antimitotic mechanism
- **Activities**: Antioxidant, anti-inflammatory, anti-cancer, and insecticidal activities

Requirement for protein (protein):
1. Preferably > 90% pure by gel electrophoresis
2. Total amount: 6.67-20 nmole protein content as measured by UV. Note: the accuracy of your protein amount is the single most important factor to obtaining an optimized drug loading. Please refer to the section Other Considerations in this manual to measure the protein amount.

Support

Customer can request a recommendation for the conjugation if the protein has a special feature, a less than 6.67 nmole of protein, or less than 20KDa MW of protein or peptide to be labeled. CellMosaic provides other accessory tools, such as buffers, standards, and reagents for PDC research. CellMosaic also provides fee-based support services to customers who need help analyzing the final conjugates by HPLC and determining the drug loading.

Protocol



Scheme 1. Schematic diagram of the workflow for preparing protein-MMAE conjugates starting with 20 nmole of protein (volume of reagents varies if the amount of protein is < 20 nmole).

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 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)

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2. Prepare Site and MMAE for Labeling Experiment

MMAE with VC-PAB is very hydrophobic. Protein-drug conjugates with VC-PAB-MMAE tend to aggregate and precipitate out from the solution. It is recommended that the labeling experiment be planned for only a few days before your other experiments.

Ensure you use personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves) while handling MMAE. Locate a clean space inside a chemical hood.

A1. Remove **Box 1** containing **MMAE** (red label), **Reagent A** (cyan label), and **Reagent B solution** (yellow label) from the -20°C freezer and warm to RT before opening the bag.

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. Check if the frozen liquid is thawed inside the **MMAE** tube. Briefly mix & spin the centrifuge tube containing **MMAE**. Place the **MMAE** tube in a tube holder inside a chemical hood and wait until the protein 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 Protein Samples for Conjugation

Items needed: Filter Devices (CM03CD050A), Collection Tube, Buffer A (CM02001, Orange label), 1.5 mL Centrifuge Tube, Clean Centrifuge Tubes (not provided in the kit).

Total amount of protein used for the conjugation is 20 nmole per reaction (protein content measured by UV).

Calculation: Amount of protein (mg) = Molecular Weight (MW) of protein x 0.00002

Reaction Scale: If you have less than 20 nmole of protein, use the calculations in **Steps B10**, **C3**, **D5**, **D6, D7, D10, E5,** and **E6** to obtain the correct volumes to be added in each step.

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

- ✓ If your protein is supplied as a lyophilized solid, dissolve the protein in 500 µL of **deionized water** and then transfer the entire contents to the **Filter Device**.
- ✓ If your protein is supplied in < 500 µL buffer, transfer your protein sample to the **Filter Device** directly. Add **Buffer A** to make up the total volume to 500 µL and cap it.

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- ✓ If the volume of your protein sample is between 500 and 1000 µL, divide the volume into two **Centrifugal Filter Devices** and add the protein sample to the filter device. Add **Buffer A** to make up the total volume to 500 µL in each device and cap them.
- ✓ If the volume of your protein sample is >1000 µL, add up to 500 µL of sample to the two **Filter Devices** and cap them. Repeat Steps **B1-B4** until all of the protein sample is transferred into the **Filter Device**. Move on to Step **B5**. Add **Buffer A** 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 10 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 10 to 20 minutes. The typical volume is ~40 µL after spinning for 10 minutes on 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 400-450 µL of **Buffer A** to make up the total volume to 500 µL. Next, 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** two more times.

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

B8. Add 20-80 µL of **Buffer A** to the **Filter Device** to rinse (actual volume of **Buffer A** added will depend upon the calculated total volume in **Step B10**). Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step B7.**

B9. Repeat **Step B8** once.

B10. Add **Buffer A** to the 1.5 mL micro-centrifuge tube from **Step B9** to make up the total volume of the sample to **290 ± 5 µL** and cap it.

Calculation 1 for Less Protein:
$Total\ volume\ of\ the\ protein\ in\ Step\ \boldsymbol{B10}\ (\mu L) = protein\ in\ nmole\ \times 14.5$

B11. Vortex the combined protein sample for 30 seconds and then spin down.

4. Thiolation of Protein (Step 1 in Scheme 1)

Items needed: Reagent A (CM12101, cyan label), Solution A (CM01003, green label), Protein Solution from **Step B11**, Ice Bath.

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

C2. Spin **Solution A** (green label) before opening it. Add 600 µL of **Solution A** to the tube with **Reagent A** from **Step C1**. Vortex for 30 seconds to 1 minute to dissolve the reagent and then spin.

C3. Add **Reagent A solution** from **Step C2** to the centrifuge tube containing protein from **Step B11**. The volume of **Reagent A solution** being transferred is adjusted based on the MW of your protein so that protein can be loaded with optimal number of thiol groups for preparing a PDC without too much aggregation according to the following list:

MW (≥60KDa): transfer **10 µL** (target 3-5 MMAE per protein)

MW (20-60KDa): transfer **8 µL** (target 2-4 MMAE per protein)

(Discard any unused **Reagent A** as hazardous chemical waste **after completion of all experiments**)

Calculation 2 for Less Protein:

MW (≥60KDa):

$$Volume\ of\ Reagent\ A\ solution\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Protein\ in\ nmole\ \times 0.5$$

MW (20-60KDa):

$$Volume\ of\ Reagent\ A\ solution\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Protein\ in\ nmole\ \times 0.4$$

C4. Vortex the solution for 30 seconds, and then spin down. Mix at RT for 1 h (**While waiting for the reaction to complete, move on to Step D1 and equilibrate the column for purification**).

Tip for mixing: You can use a nutator, a shaker, a vortex, or an incubator shaker for mixing. If you are using end to end nutating, make sure the tube from **step C4** is securely capped. If you don't have any of this equipment, you can let the tube sit on the bench with manual mixing by pipetting every 20 minutes.

5. Purification to Remove Excess Reagent A and Conjugation with MMAE (Step 2)

The following steps are to be performed without any break. Free thiols tend to oxidize quickly. Make sure **step A3** is completed prior to the following steps. Work quickly through **steps D4-D8**.

Items needed: Desalting Column 1 (CM03SG05), Collection Tube, Buffer B (CM02005, indigo label), MC-VC-PAB-MMAE (CM11001, red label), Reagent B Solution (CM12004.1, yellow label), 1.5 mL Centrifuge Tube, Protein Solution from **Step C4**.

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D1. In a chemical hood, securely attach **Desalting Column 1** to a support stand, a 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 3 mL of **Buffer B** and allow the buffer to completely enter the gel bed by gravity flow.

D3. Repeat **Step D2** two times.

Work quickly

D4. Add the sample from **step C4** to the column. Allow the sample to enter the gel bed completely.

D5. Add 200 µL of **Buffer B** and allow the buffer to completely enter the gel bed by gravity flow (**Note:** this elution buffer does not contain any of your product, you can let it drain to the waste).

Calculation 3 for Less Protein:

$$Volume\ of\ Buffer\ B\ in\ Step\ \mathbf{D5}\ (\mu L) = 500 - protein\ in\ nmole\ \times 15$$

D6: Open the tube containing **MMAE** from **Step A3** and place it directly under the column. If you have less protein, transfer the calculate amount of **MMAE** solution from **Step A3** to a clean 1.5 mL centrifuge tube and place it directly under the column.

Calculation 4 for Less Protein:

$$Volume\ of\ MMAE\ Solution\ to\ be\ Transferred\ in\ Step\ \mathbf{D6}\ (\mu L) = protein\ in\ nmole\ \times 5.5$$

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

D7: Add 800 µL of **Buffer B** to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

Calculation 5 for Less Protein:

$$Volume\ of\ Buffer\ B\ in\ Step\ \mathbf{D7}\ (\mu L) = 500 + protein\ in\ nmole\ \times 15$$

D8. Cap the centrifuge tube. Mix at 25°C or RT for 1 h.

D9. Spin the centrifuge tubes containing **Reagent B solution** (Yellow label).

D10. Spin the MMAE-labeled protein solution from **Step D8** before opening it. Add **10 µL** of **Reagent B solution** from **Step D8** to the centrifuge tube (Discard any unused **Reagent B** as hazardous chemical waste **until the experiments are done**).

Calculation 6 for Less Protein:

$$Volume\ of\ \mathbf{Reagent\ B\ solution}\ to\ be\ Transferred\ in\ Step\ \mathbf{D10}\ (\mu L) = protein\ in\ nmole\ \times 0.5$$

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D11. Vortex the solution for 30 seconds, and then spin down. Mix at RT for 30 minutes.

> **Time-saving tip**: While waiting for the reaction to complete, you can move on to **Step E1** and equilibrate the column for purification.

6. Purification of Conjugate

Items needed: Desalting Column 2 (CM03SG10), Storage Buffer (1x PBS), 2.0 mL Centrifuge Tube, Hazardous Waste Bag, Protein Solution from **Step D11**.

E1. In a chemical hood, securely attach the **Desalting Column 2** 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.
E2. Add 5 mL of **Storage Buffer** and allow the buffer to completely enter the gel bed by gravity flow.
E3. Repeat **Step E2** twice.
E4. Spin the MMAE-labeled protein solution from **Step D11** before opening it. Add the entire protein solution to the column. **Dispose of the centrifuge tube in the hazardous waste bag.**
E5. Add 200 µL of **Storage Buffer** and allow the liquid to enter the gel bed completely (**Note:** this elution does not contain any of your product, you can let it drain to the waste).

> **Calculation 7 for Less Protein**:
> $$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{E5}\ (\mu L) = 1000 - protein\ in\ nmole\ \times 40$$

E6. Place a 2 mL centrifuge tube under the column. Add 1.3 mL of **Storage Buffer** to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

> **Calculation 8 for Less Protein**:
> $$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{E6}\ (\mu L) = 500 + protein\ in\ nmole \times 40$$

E7. Label the tube as your product. Store your conjugate at 4°C. **Dispose of the Desalting Column in the hazardous waste bag and seal the bag. Dispose of the waste following regulations appropriate for your area.**

E8. Determine the concentration and the estimated DPR by UV/Vis spectrophotometry (see other considerations).
E9. The protein-MMAE labeled here is relatively stable at 2-8°C for a few weeks. For long-term storage, add **Stabilization PBS buffer (5x)** (Pink label) to the protein-MMAE from **Step E7**. Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness.

Conjugate is Ready for Your Experiment

- **Specifications of your product:** A typical batch contains more than 95% conjugated products by size exclusion chromatography (SEC) with less than 5% unreacted protein and is free of any unreacted drug. The approximate concentration of the PDC is 7.7 µM in PBS buffer assuming 50% recovery. You can determine the concentration and estimated DPR of the PDC by UV/vis spectrophotometry (see Other Considerations).

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Other Considerations

1. Concentration Determination for Protein (Unlabeled)

The accuracy of the protein amount is important for obtaining an optimized drug loading in this protocol. The simplest assay method for determining protein concentration in solution is to measure the absorbance of the protein at 280 nm if you know the extinction coefficient of your protein. Otherwise you can use a protein assay such as BCA to determine the concentration.

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

$$Concentration\ (M)\ of\ protein = \frac{(A280)}{\mathcal{E} \times L}$$

Where **L** is the UV cell path length (cm) and **Ɛ** is the extinction coefficient of your protein ($cm^{-1}M^{-1}$)

If your protein comes with a buffer that has UV absorbance at 280 nm, you can determine the concentration in **step B11** after exchanging it with reducing buffer and assuming **95%** recovery of the protein after buffer exchange. Buffer A does not contain any substances that will interfere with the UV measurement at 280 nm. The total volume of Buffer A added in **Step B10** can be estimated based on the initially estimated amount of protein and will not affect the conjugation too much if the volume is off to some extent.

$$Concentration\ (M)\ of\ Starting\ Protein = \frac{(A280)}{\mathcal{E} \times L \times 0.95}$$

After calculating the total amount, follow the calculations in **Steps B10**, **C3**, **D5**, **D6, D7, D10, E5,** and **E6** to obtain the correct volumes to be added in each step.

2. Concentration Determination for PDC

To determine the concentration of the PDC, dilute your conjugate from **Step E7** 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 formula:

$$Concentration\ (\mu M)\ of\ the\ dilute\ sample = \frac{(A280) * 1000000}{L\ (\mathcal{E}\ + n * 1425)}$$

$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280) \times 150000}{L(\mathcal{E}\ + n * 1425)}$$

Where **L** is the UV cell path length (cm). If you are using a 1 cm UV cell, you can dilute the conjugate 2-4 times to obtain a good reading.

Where **n** is the average molar ratio of MMAE per protein. If you do not have the experimental value of your conjugates, use **2** for protein with MW of 20-60KDa, **3** for protein with MW ≥60KDa

The molar extinction coefficient of Mc-VC-PAB-MMAE after conjugation is ~1425 $M^{-1}cm^{-1}$ measured and calculated at CellMosaic.

3. MW Calculation for PDC

Calculation of the MW of the conjugate:

MW(PDC) = n x 1317+ MW(Protein)

Where n is the average molar ratio of MMAE per protein. Use **2** for protein with MW of 20-60KDa, **3** for protein with MW ≥60KDa.

4. Drug-to-Protein Ratio (DPR) and Characterization by UV and MS

A) If you know the extinction efficient of your protein at 280 and 248 nm, you can use UV to determine the DPR. To estimate the DPR, you first have to obtain the UV absorbance ratio (R) of your conjugate at 248 nm and 280 nm.

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

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

$$DAR = \frac{(E280nm\ of\ protein \times R - E252nm\ of\ protein)}{(16150 - 1425 \times R)}$$

DM1: $E_{280\ nm}$ = 1425 $M^{-1}cm^{-1}$ and $E_{252\ nm}$ = 16150 $M^{-1}cm^{-1}$ (measured at CellMosaic)

Note: The UV absorbance of the MMAE in an PDC can vary greatly depending on many factors, such as aggregation and stacking. Therefore, the **R** value for an PDC can differ greatly for different proteins and should be determined experimentally. The calculation for the DPR using this formula is for reference only.

B) If you don't know the extinction efficient of your protein, we strongly recommend sending your sample for an intact MS analysis (either MALDI-TOF MS or LC-MS will be fine). By comparing the intact MS of PDC and unlabeled protein, you can calculate the average DPR. If you do not have access to a MS facility, please contact CellMosaic for analysis.

5. Characterization of PDC by HIC HPLC

For PDCs prepared via surface amines of the protein, hydrophobic interaction chromatography (HIC) HPLC can be used to check if a protein is labeled or not. However, due to the highly heterogeneous nature of surface amine labeling, protein loaded with the same number of drugs (same DPR) may have slightly different hydrophobicity. For a typical MMAE PDC, a broad peak will be seen without clear separation of the peaks.

CellMosaic offers an HIC buffer set (Product #: CM02025) for our customers to use with any HIC column. The CM02025 product sheet contains all of the information and methodology needed to run an HIC HPLC analysis.

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

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6. Characterization of PDC by SEC HPLC

VC-PAB-MMAE is very hydrophobic. This kit is designed to minimize the aggregation and precipitation issues generally seen with MMAE labeling. However, you may still notice some solid precipitate out or PDC aggregation during the reaction. The precipitate will be removed during purification. Depending on the properties of your protein, recovery will be 40-80%. If you are concerned with the aggregation, you can use size exclusion chromatography (SEC) to check the extent of aggregation. SEC separates the conjugates by apparent molecular weight (MW) or size in aqueous solution. The larger the MW of the conjugate, the earlier it elutes. By comparing the SEC profile of unlabeled protein and the PDC, you can estimate how much aggregation is in the PDC.

CellMosaic offers two SEC standards (Product #: CM92004 and CM92005) for our customers to use with any SEC column. The CM92004 product sheet contains all of the information and methodology you need to run 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 to prevent the hydrophobic drugs from interacting with each other during storage, which would cause the conjugates to precipitate out. Stabilization buffer also helps preserve the structure of the PDC during lyophilization. The buffer is biocompatible and can be used directly for any *in vitro* and *in vivo* studies. For more information on the stabilization buffers, please check our website.

8. Recommended Storage Conditions

PDC with MMAE is relatively stable. Based on our preliminary data, the conjugate made with this kit can remain stable in PBS buffer for several weeks at 2-8°C. Do not freeze MMAE PDC.

The stability of your conjugate may be different due to your protein and should be checked by either HPLC or UV. If you need to store the PDCs for a longer period of time, dilute your PDC 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.

9. Sample Submission for HPLC Analysis

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

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 un-conjugated protein in PBS buffer to 1 mg/mL, and then transfer 50 µL of the diluted solution to a 500 µL micro-centrifuge tube. Label the vial properly.

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

3) Transfer 50 µL of PDC (non-diluted solution) to a 500 µL micro-centrifuge tube and label the vial properly.
4) Ship your samples with a cold pack for overnight delivery.

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Appendix: Examples of MMAE PDC

Example 1: MMAE Conjugation with Bovine Serum Albumin (BSA)

Kit Lot number: 5529.S14.072519

Scale of the reaction: 20 nmole following the exact protocol without any adjustment. Data provided for information only.

Specification of the final conjugates:

Calculated average DPR based on the A248 nm and A280 nm ratio: ~4

Calculated average DPR based on the MS data: ~3

% of unreacted antibodies: 0%; % of unreacted MMAE: 0%; Product yield: 65%

Figure 1: HIC HPLC analysis of BSA (blue trace) and purified conjugates (red trace). MMAE is labeled at the protein via surface amines. The conjugate is very heterogeneous and appears as a very broad peak.

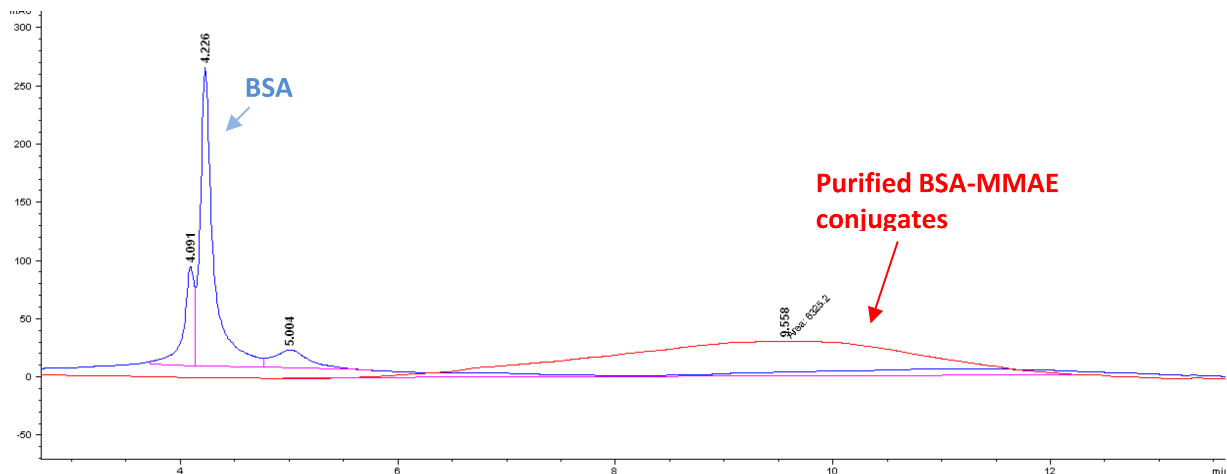

Figure 2: SEC/UV HPLC analysis of BSA (green trace) and purified conjugates (purple trace).

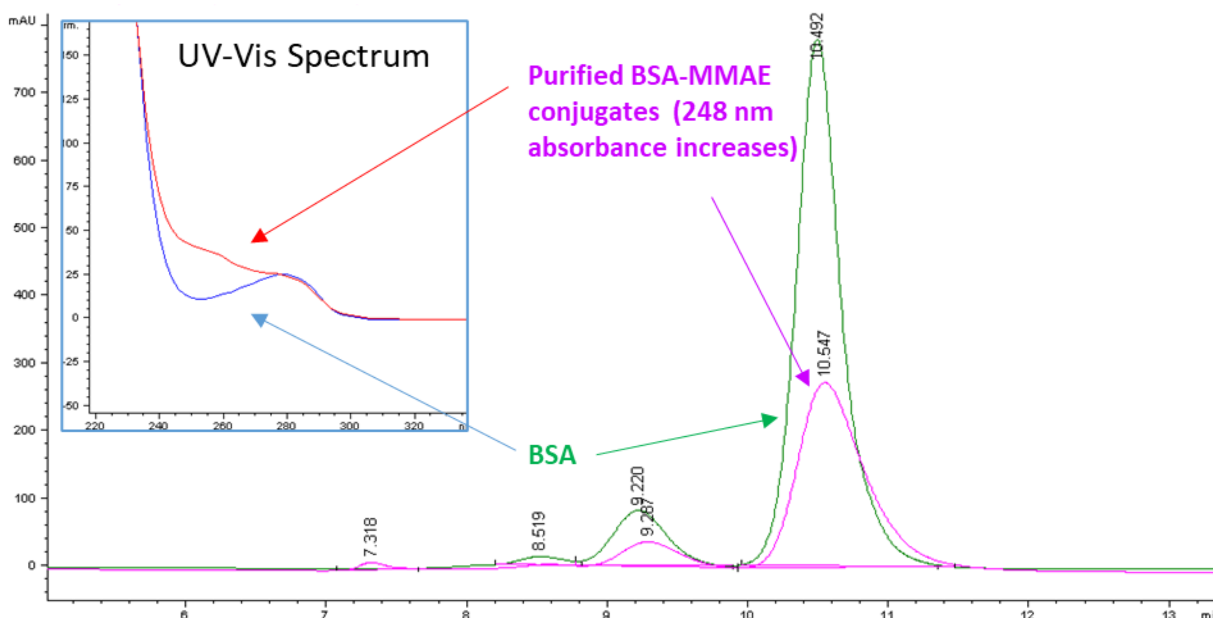

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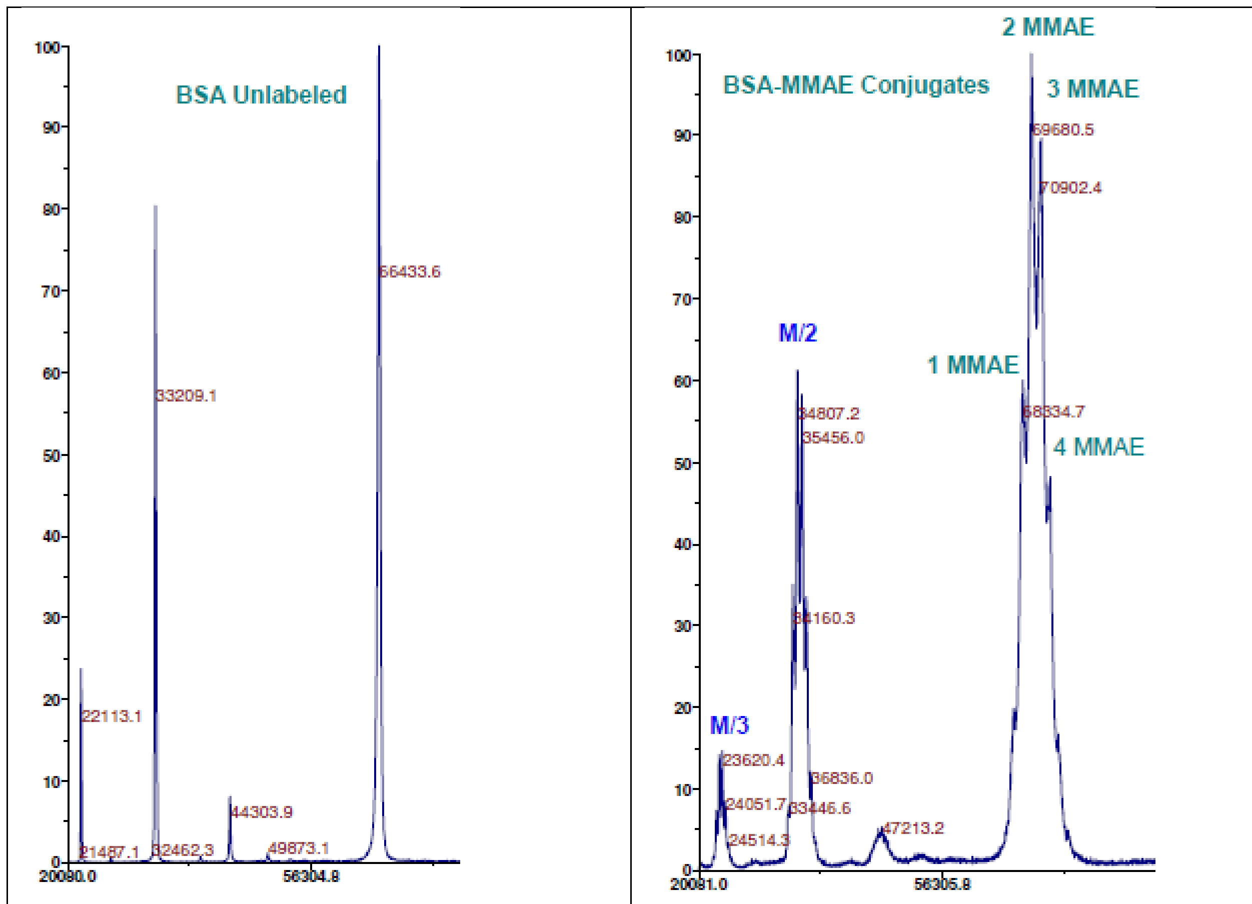
Figure 3: MS analysis of BSA (left) and purified conjugates (right) (data acquired by MDS SCIEX 4800 MALDI TOF Analyzer).



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Example 2: Fab MMAE conjugation

Fab information: Fab was prepared following a similar protocol as CellMosaic's PerKit™ Fab preparation kit (Cat#: CM51407) from a rabbit IgG antibody.

Kit Lot number: 5529.S14.072519

Scale of the reaction: 2 nmole (limited material available) following a modified protocol. Data provided for information only.

Figure 4: HIC HPLC analysis of Fab and purified conjugates

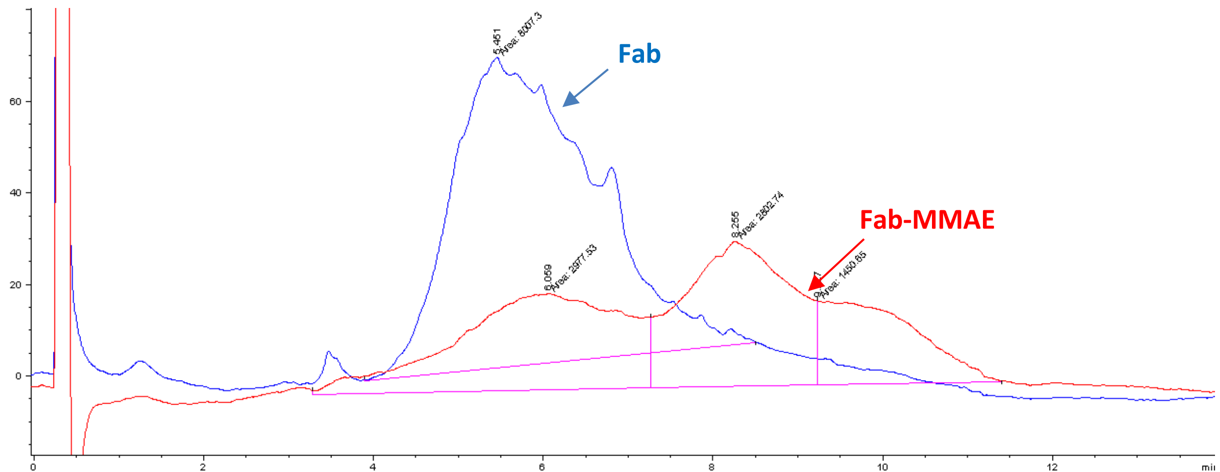

Figure 5: SEC HPLC analysis of Fab and purified conjugates