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PerKit™ Protein DM1 Conjugation Kit (CM11414x1 and CM11414x3) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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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 nano-mole (nmol) of one (CM11414) or three (CM11414x3) protein samples (MW ≥ 20KDa) with mertansine (DM1) using SMCC (succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate) 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 (CM11414)	Quantity (CM11414x3)	Storage condition
Box 1	DM1 (red label)	CM11002	1 unit	3 units	-20°C
	SMCC (green label)	CM12104	1 unit	3 units	
Box 2	Solution A (blue label)	CM01006	0.3 mL	0.5 mL	2-8°C
	Buffer A (orange label)	CM02001	4 mL	12 mL	
	Buffer B (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 Device	CM03CD010A	3	9	
	Desalting Column	CM03SG10	1	3	
	Collection Tubes	CM03CT0	6	18	
	1.5 mL Centrifuge Tube	CM03CT2	3	9	
	Hazardous Waste Bag	CM03HZ1	1	3	
User Material	Protein (MW: ≥20 KDa)	N/A	NOT PROVIDED (User Supplied Material, **6.67-20 nmol** needed per reaction)		

Reaction Scale: The protocol is optimized for conjugating 20 nmol of protein. If you have less than 20 nmol of protein, use the calculations in **Steps B10**, **C3**, **D10**, **E3**, **F5,** and **F6** 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.

Labeling Chemistry

The kit is designed to label any protein with mertansine (DM1) using SMCC linker. The user supplies the protein. This kit includes SMCC and DM1, which can be coupled to the protein sequentially via surface

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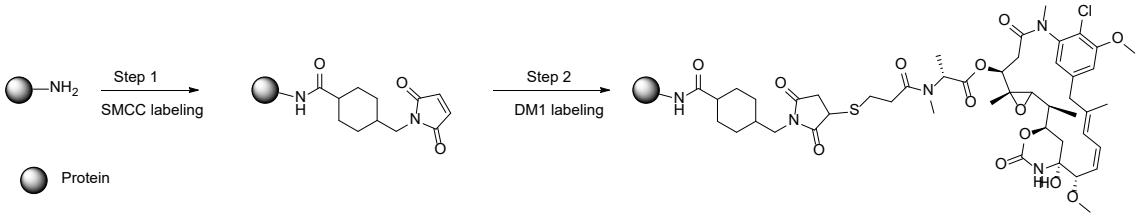
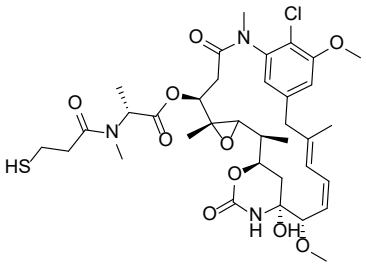
amines (a method developed by Immunogen). The product is then purified to remove any unreacted drug.



Key features of this conjugation kit:

- Offers a simple and easy way to label protein with DM1 with minimum exposure to the toxin
- Stable linkage
- Fast and easy preparation: 6 h preparation and <2 h hands-on time
- All reagents and supplies included for preparation and purification
- Loading is optimized based on the MW of the protein
- Less than 5% of protein aggregation and >99% of conjugated products by SEC (size-exclusion chromatography)

Drug Information:



- **Name**: Mertansine (DM1)
- **CAS number**: 139504-50-0
- **Chemical Formula**: $C_{35}H_{48}ClN_3O_{10}S$
- **MW**: 738.29
- **Mechanism of action**: Inhibits cell division by blocking the polymerization of tubulin

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

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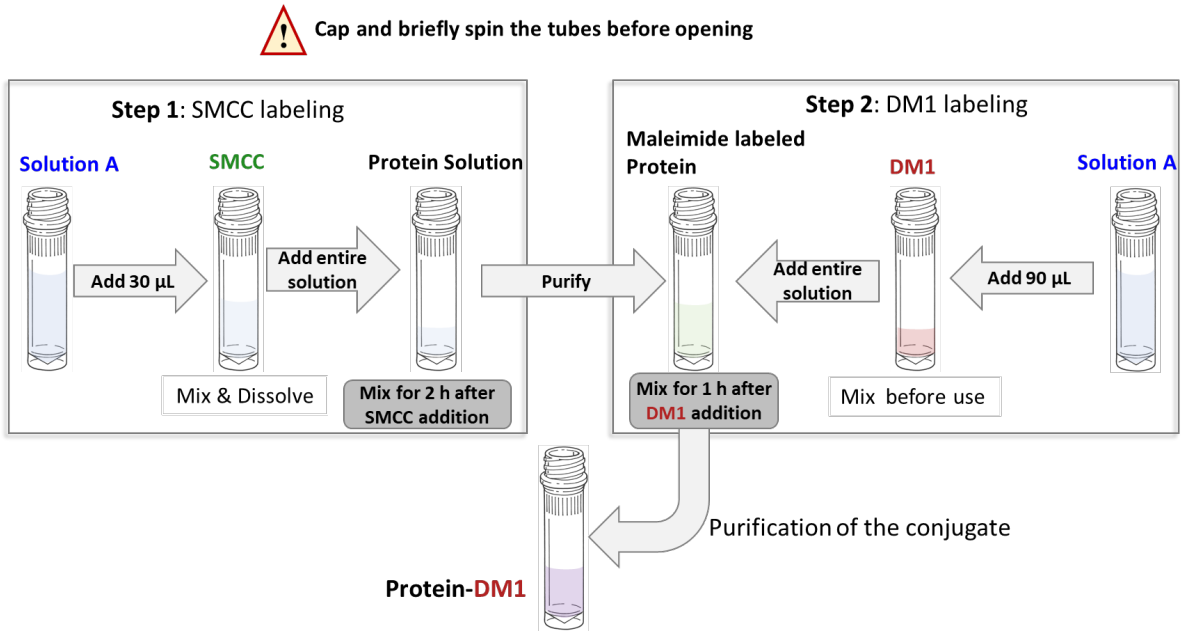
Support

Customer can request a recommendation for the conjugation if the protein has a special feature, a less than 6.67 nmol 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 protein drug conjugate (PDC) research. CellMosaic also provides fee-based support services to customers who need help analyzing the final conjugates by HPLC.

Protocol



Scheme 1. Schematic diagram of the workflow for preparing protein-DM1 conjugates starting with 20 nmol of protein (volume of reagents varies if the amount of protein is < 20 nmol).

1. Lab Instrumentation Needed

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

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2. Prepare Site and Reagents for Labeling Experiment

> **Note:** DM1 and SMCC are very hydrophobic. PDC with DM1 via SMCC linker tend to aggregate and precipitate out from the solution over time. It is recommended that the labeling experiment be planned for only a few days before your other experiments. If not possible, then please use the stabilization PBS buffer to store under recommended conditions.

Ensure you use personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves) while handling DM1. Locate a clean space inside a chemical hood.

A1. Remove **Box 1** containing **DM1** (red label) and **SMCC** (green 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. Briefly spin the centrifuge tube containing **DM1**. Place the **DM1** 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 (CM03CD010A), Collection Tubes (CM03CT0), Buffer A (CM02001, orange label), 1.5 mL Centrifuge Tube (CM03CT2), Clean Centrifuge Tubes (not provided in the kit).

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

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

> **Reaction Scale:** If you have less than 20 nmol of protein, use the calculations in **Steps B10**, **C3**, **D9**, **E2**, **F5,** and **F6** to obtain the correct volumes to be added in each step.

B1. Insert the **Filter Device** into one of the provided collection tubes (microcentrifuge 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 estimate the approximate volume of the concentrated sample).

B8. Add 40 to 100 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 **570 ± 5 µL** and cap it.

Calculation 1 for Less Protein:

$$Total\ volume\ of\ the\ protein\ in\ Step\ \boldsymbol{B10}\ (\mu L) = protein\ in\ nmol\ \times 28.5$$

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

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4. SMCC Labeling (Step 1 in Scheme 1)

Items needed: SMCC (CM12104, green label), Solution A (CM01006, blue label), Protein Solution from **Step B11**.

C1. Spin the centrifuge tube containing **SMCC** (green label) before opening it.

C2. Spin **Solution A** (blue label) before opening it. Add **30 µL** of **Solution A** to the **SMCC** tube from **Step C1**. Vortex for 30 seconds to 1 minute to dissolve the reagent and then spin the centrifuge tube before opening it (Solution **A** will be also used in **Step E2**).

Tip for solubility check: Check the bottom of the micro-centrifuge tube to see if the solution is clear of any solid residue.

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

MW (≥80KDa): transfer **30 µL** (target 3-5 maleimide groups per protein)

MW (40-80KDa): transfer **20 µL** (target 2-4 maleimide groups per protein)

MW (20-40KDa): transfer **15 µL** (target 1-3 maleimide groups per protein)

Calculation 2 for Less Protein:

MW (≥80KDa):

$$Volume\ of\ SMCC\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Protein\ in\ nmol\ \times 1.5$$

MW (40-80KDa):

$$Volume\ of\ SMCC\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Protein\ in\ nmol\ \times 1.0$$

MW (20-40KDa):

$$Volume\ of\ SMCC\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Protein\ in\ nmol\ \times 0.75$$

Note: Maleimide loading may vary for individual protein (e.g. the number of accessible surface amines will affect the loading).

C4. Vortex the solution for 30 seconds, and then spin down. Mix at 25 °C or RT for 2 h.

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

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5. Purification to Remove Excess SMCC

Items needed: Filter Device (CM03CD050A), Collection Tubes (CM03CT0), 1.5 mL Centrifuge Tube (CM03CT2), Buffer B (CM02005, indigo label), Clean Centrifuge Tubes (not provided in the kit), Protein Solution from **Step C4**.

D1. Insert the **Filter Device** into one of the provided collection tubes (microcentrifuge tube with the cap attached). Transfer the entire SMCC labeled protein solution from **Step C4** into the **Filter Device** directly. Add **Buffer B** to make up the total volume to 500 µL if the total volume of protein solution is less than 500 µL. Place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

D2. Spin the **Filter Device** at 14,000 x g for 10 minutes (preferably cooled to 4°C) to concentrate to < 100 µL.

D3. 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.**

D4. Insert the **Filter Device** back to the collection tube. If there is any leftover SMCC labeled protein solution from **Step C4**, transfer the rest of the solution directly into the **Filter Device**. Otherwise, go directly to **Step D5**. Add **Buffer B** to make up the total volume to 500 µL. Spin the device at 14,000 x g to concentrate to **< 100 µL**. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

D5. Insert the **Filter Device** back to the collection tube. Add 400-450 µL of **Buffer B** to make up the total volume to 500 µL. Spin the device at 14,000 x g to concentrate to **< 100 µL**. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

D6. Repeat **Step D5** two more times.

D7. 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).

D8. Add 30 to 100 µL of **Buffer B** to the **Filter Device** to rinse (actual volume of **Buffer B** added will depend upon the calculated total volume in **Step D10**). Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step D7.**

D9. Repeat **Step D8** once.

D10. Add **Buffer B** to make up the total volume of the sample to **510 ± 5 µL** and cap it.

Calculation 3 for Less Protein:

$$Total\ Volume\ of\ SMCC\ Labeled\ Protein\ in\ Step\ \boldsymbol{D10}\ (\mu L) = Protein\ in\ nmol\ \times 25.5$$

D11. Vortex the combined protein sample for 30 seconds and then spin down.

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Note: If necessary, you can purchase the maleimide assay kit (Product#: CM90002) separately from CellMosaic to assay the maleimide content. The kit is simple and easy to use. Mix 7 µL of the protein solution from **Step D11** with 63 µL of **Buffer A** (included in the maleimide assay kit) for the assay following the protocol provided with the maleimide assay kit. Final concentration of the protein is 39.2 µM if the total volume of the protein is 510 µL (prior dilution).

6. DM1 Labeling (Step 2 in Scheme 1)

Items needed: DM1 (CM11002, red label), Solution A (CM01006, blue label), Hazardous Waste Bag (CM03HZ1), Protein Solution from **Step D11**.

E1. With personal protection equipment on, carefully open the centrifuge tube of DM1 from **Step A3**.

E2. Add **90 µL** of **Solution A** to the **DM1** tube. Vortex for 30 seconds to 1 minute to dissolve the reagent and then spin down.

E3. Transfer the entire **DM1 solution** from **Step E2** to the centrifuge tube containing protein from **Step D11**. When you add the DM1 solution, place the pipette tip inside the protein solution and then dispense the DM1 slowly while swirling the pipette tip. **Dispose of the pipette tip and DM1 tube in the solid waste bag**.

Calculation 4 for Less Protein:

$$Volume\ of\ DM1\ to\ be\ Transferred\ in\ Step\ \boldsymbol{E3}\ (\mu L) = Protein\ in\ nmol\ \times 4.5$$

Dispose of the remainder of the DM1 solution in the hazardous waste bag.

E4. Cap the centrifuge tube. Mix at 25°C or RT for 1 h.

Time saving tip: While waiting for the reaction to complete, you can move on to **Step F1** and equilibrate the column for purification.

7. Purification of Conjugate

Items needed: Desalting Column (CM03SG10), Storage Buffer (1x PBS, CM02013, grey label), ADC Stabilizing PBS Buffer (5x) (CM02022, pink label), 1.5 mL Centrifuge Tube (CM03CT2), Hazardous Waste Bag (CM03HZ1), Protein Solution from **Step E4**.

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 and allow the excess liquid to flow through by gravity. Collect the liquid in a flask.

F2. Add 5 mL of **Storage Buffer** and allow the buffer to completely enter the gel bed by gravity flow.

F3. Repeat **Step F2** twice times.

F4. Spin the DM1 labeled protein solution from **Step E4** before opening it. Add the entire protein solution to the column. Allow the sample to enter the gel bed completely. **Dispose of the centrifuge tube in the solid waste bag.**

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F5. Add 400 µL of **Storage Buffer** and allow the liquid to enter the gel bed completely (**Note:** this elution buffer does not contain any of your product, you can let it drain to the waste).

Calculation 5 for Less Protein:

$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F5}\ (\mu L) = 1000 - Protein\ in\ nmol\ \times 30$$

F6. Place a 1.5 mL centrifuge tube under the column. Add 1.1 mL of **Storage Buffer** to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

Calculation 6 for Less Protein (Ab):

$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F6}\ (\mu L) = 500 + Protein\ in\ nmol\ \times 30$$

F7. Label the tube as your product. Store your conjugate at 4°C. **Dispose of the Desalting Column in the solid waste bag and seal the bag. Dispose of the waste following regulations appropriate for your area.**

F8. Determine the concentration and the estimated DAR by UV/Vis spectrophotometry (see other considerations).

F9. If the PDC is not used immediately for the experiment, add **Stabilization PBS buffer (5x)** (pink label) to the PDC from **Step F7**. Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness for long-term storage.

Conjugate is Ready for Your Experiment

- **Specification for your product:** A typical batch contains more than 99% conjugated products by size exclusion chromatography (SEC) and is free of any unreacted drug. The approximate concentration of the PDC is 9 µM in PBS buffer assuming 50% recovery. You can determine the concentration and estimated DAR of the PDC by UV/Vis spectrophotometer (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 to Protein Ratio (DPR) in this protocol. The simplest assay method for determining protein concentration in solution is to measure the absorbance of the protein at 280 nm (UV range) 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.

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

Where **L** is the UV cell path length (cm) and **ε** 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 Buffer A 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.

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

After calculating the total amount, follow the calculations in **Steps B10, C3**, **D10**, **E3**, **F5,** and **F6** to obtain the correct volumes to be added in each step.

2. Concentration Determination for Protein Drug Conjugate (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:

$$Concentration\ (\mu M) of\ the\ dilute\ sample = \frac{(A280) * 1000000}{L\ (\varepsilon + n * 5700)}$$

$$Concentration\ (mg/mL) of\ the\ dilute\ sample = \frac{(A280) \times 150000}{L(\varepsilon + n * 5700)}$$

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.

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Where **n** is the average molar ratio of DM1 per protein. If you do not have the experimental value of your conjugates, use **2** for protein with MW of 20-40KDa, **3** for protein with MW of 40-80KDa, and **4** for protein with MW ≥80KDa.

The molar extinction coefficient for DM1 is 5700 $M^{-1}cm^{-1}$ (Ravi V.J. Chari *et. al.* Immunoconjugates containing novel maytansinoids: promising anticancer drugs. *Cancer research* **1992**, *52*, 127-131.)

3. MW Calculation for PDC

Calculation of the MW of the conjugate:

MW(PDC) = n x 957+ MW(Protein)

Where **n** is the average molar ratio of DM1 per protein. Use **2** for protein with MW of 20-40KDa, **3** for protein with MW of 40-80KDa, and **4** for protein with MW ≥80KDa.

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

There are various ways you can determine the Drug-to-Protein Ratio (DPR).

A) If you perform the maleimide assay in step D11 using Maleimide Assay Kit (Product#: CM90002), your DPR will be very close to the maleimide load.

B) If you know the extinction efficient of your protein at 280 and 252 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 252 nm and 280 nm.

$$R = {(A252)}/{(A280)}$$

Then you can use the following formula to calculate the estimated DPR (for reference only):

$$DAR = \frac{(E280nm\ of\ protein \times R - E252nm\ of\ protein)}{(28044 - 5700 \times R)}$$

DM1: $E_{280\ nm}$ = 5700 $M^{-1}cm^{-1}$ and $E_{252\ nm}$ = 28044 $M^{-1}cm^{-1}$(Extinction coefficient values of DM1 at 252 nm were taken from this reference: Ravi V.J. Chari *et. al.* Immunoconjugates containing novel maytansinoids: promising anticancer drugs. *Cancer research* **1992**, *52*, 127–131.)

Note: The UV absorbance of the DM1 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 DAR using this formula is for reference only

C) 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

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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 DM1 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.

6. Characterization of PDC by SEC HPLC

DM1 is very hydrophobic. This kit is designed to minimize the aggregation and precipitation issues generally seen with DM1 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 30-60%. 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 any 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.

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8. Recommended Storage Conditions

PDC with DM1 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 DM1 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. Submit Samples 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.
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.