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# PerKit™ F(ab')2 MMAE Conjugation Kit (CM11416x1 and CM11416x3) User Reference Guide

## Contents

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# Important Notes & Contact Information

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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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## Kit Components

This kit provides materials to conjugate 0.73 to 2.2 mg of a single F(ab')2 sample (CM11416x1) or three F(ab')2 samples (from **IgG**) (CM11416x3) 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 (CM11416x1) | Quantity (CM11416x3) | Storage condition |
|---|---|---|---|---|---|
| **Box 1** | MC-VC-PAB-MMAE (**red label**) | CM11001 | 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 | |
| | Centrifugal Filter Devices | CM03CD030A | 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 F(ab')2 | N/A | NOT PROVIDED (User Supplied Material, **0.73-2.2 mg F(ab')2** needed per reaction) | | |

**Reaction Scale:** The protocol is optimized for conjugating 2.2 mg of IgG F(ab')2. If you have less than 2.2 mg of F(ab')2, use the calculations in **Steps B10**, **C3**, **D9**, **E2**, **F5,** and **F6** to obtain the correct volumes to be added in each step.

**Drug-to-F(ab')2 Ratio (DAR) Optimization:** The reducing protocol is optimized for F(ab')2 fragmented from monoclonal IgG1 subtype to obtain an average 2-4 thiols per F(ab')2. For F(ab')2 fragmented from other IgG subtypes or polyclonal antibodies, the DAR may vary. For the best performance of the ADC and to obtain the desired DAR, you can purchase the Thiol Assay Kit with Purification (Product #: CM90005) separately and use it to perform an in-process thiol assay after the F(ab')2 reduction (**Step C5 Note Section**). The amount of reducing reagent can be adjusted based on the data to obtain your desired DAR.

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## 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.

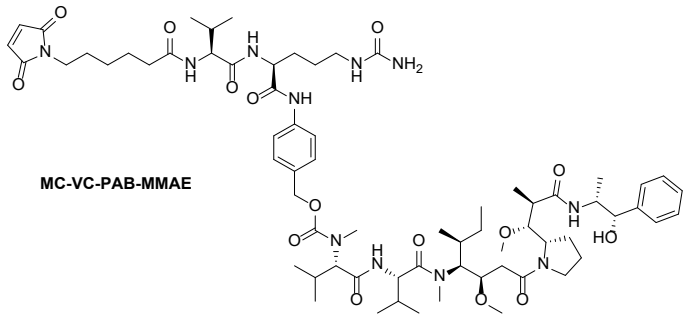
## Labeling Chemistry

The kit is designed to label any F(ab')2 with monomethyl auristatin E (MMAE) using a valine-citruline p-aminobenzylcarbamate (VC-PAB) linker. The user supplies the F(ab')2. The kit includes maleimide-activated VC-PAB-MMAE, which can be coupled directly to the F(ab')2 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 purified to remove any unreacted drugs.

Key features of this conjugation kit:

- Simple and efficient labeling of F(ab')2 with MMAE, minimizing toxin exposure
- Features Cathepsin B cleavable VC-PAB linker (Ref. Doronina *et al*. **2008**, *Bioconjugate Chem*. 19, 1960-1963)
- Fast preparation: 6 h preparation and <2 h hands-on time
- Includes all necessary reagents and supplies for preparation and purification
- Easy to control the DAR if used together with the Thiol Assay Kit with Purification (Product #: CM90005)
- Over 95% conjugated products (free of unreacted drug and less than 5% of unreacted F(ab')2)
- 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

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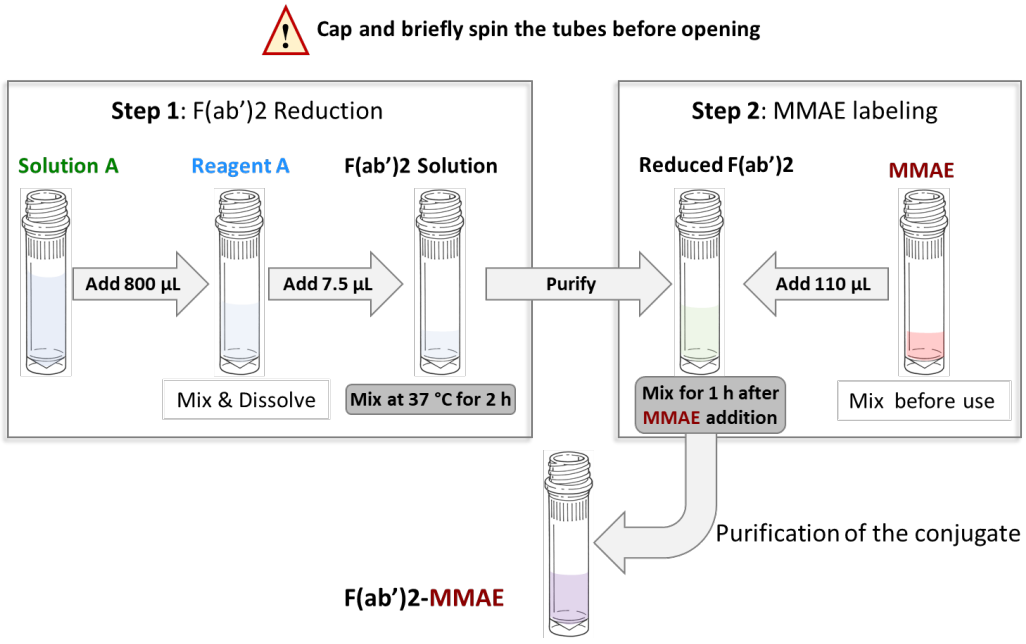
- **Mechanism of action**: Inhibits cell division by blocking the polymerization of tubulin. The VC-PAB linker is stable in extracellular fluid but cleaved by cathepsin B once inside the tumor cell, activating the antimitotic mechanism.
- **Activities**: Antioxidant, anti-inflammatory, anticancer, and insecticidal activities.

**Requirement for F(ab')2:**
1. Preferably > 90% pure by gel electrophoresis
2. Total amount: 0.73-2.2 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. 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 the F(ab')2 has unique features or if they need to label less than 0.73 mg of F(ab')2. CellMosaic provides additional accessory tools, such as buffers, standards, and reagents for ADC research. We also offer fee-based support services to customers who need assistance with final conjugate analysis by HPLC and determining the DAR.

## Protocol




**Scheme 1**. Schematic workflow diagram for preparing F(ab')2-MMAE conjugates, starting with 2.2 mg of F(ab')2 (Reagent volume will vary if the amount of F(ab')2 is less than 2.2 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 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 F(ab')2-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.

Always use personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves) when handling MMAE. 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 F(ab')2 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 F(ab')2 Samples for Conjugation

Items needed: Filter Devices (CM03CD030A), Collection Tube, Reducing Buffer (CM02001, Orange label), 1.5 mL Centrifuge Tube (CM03CT2), Clean Centrifuge Tubes (not provided in the kit).

The total amount of F(ab')2 used for the conjugation is 2.2 mg per reaction (protein content as measured by UV). The protocol is optimized for F(ab')2 fragmented from the monoclonal IgG1 subtype F(ab')2 to obtain an average of 4 drugs per F(ab')2.

> **Reaction Scale:** If you have less than 2.2 mg of F(ab')2, refer to the calculations in **Steps B10**, **C3**, **D9**, **E2**, **F5,** and **F6** to obtain the correct volumes to add at each step.

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**DAR Optimization:** If you have F(ab')2 fragmented from non-IgG1 subtype or polyclonal F(ab')2 and would like to adjust the loading, follow **Step C5 Note Section** for optimization.

**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 F(ab')2.

- ✓ **Lyophilized F(ab')2**: Dissolve the F(ab')2 in 500 µL of **deionized water** and transfer the entire contents to the **Filter Device**.
- ✓ **F(ab')2 in < 500 µL buffer**: Transfer the F(ab')2 sample directly to the **Filter Device**, then add **Reducing Buffer** to bring the total volume to 500 µL. Cap the device.
- ✓ **F(ab')2 in 500-1000 µL buffer**: Split the sample between two **Centrifugal Filter Devices,** adding the F(ab')2 to each device. Add **Reducing Buffer** to bring the volume in each device to 500 µL and cap them.
- ✓ **F(ab')2 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 F(ab')2 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.

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**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 F(ab')2:**

$$Total\ volume\ of\ the\ F(ab')2\ in\ Step\ \boldsymbol{B10}\ (\mu L) = F(ab')2\ in\ mg\ \times 137$$

**B11**. Vortex the combined F(ab')2 sample for 30 seconds and then spin down.

## 4. F(ab')2 Reduction (Step 1 in Scheme 1)

Items needed: Reagent A (CM13004, blue label), Solution A (CM01003, green label), F(ab')2 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 800 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 **7.5 µL** of **Reagent A solution** from **Step C2** to the centrifuge tube containing the F(ab')2 from **Step B11** (Dispose of any unused **Reagent A** as hazardous chemical waste **once all experiments are done**).

**Calculation 2 for Less F(ab')2:**

$$Volume\ of\ Reagent\ A\ solution\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = F(ab')2\ in\ mg\ \times 3.42$$

**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 2 h.

**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.

**C5**. Cool the reduced F(ab')2 solution to approximately 4°C by placing the tube on ice or keeping it inside a refrigerator at 2-8°C for 5 minutes.

**Note: Optimization of Thiol Content for F(ab')2 fragmented from non-IgG1 Subtype F(ab')2 or Polyclonal F(ab')2**

For F(ab')2 from the monoclonal IgG1 subtype, the average free thiol groups per F(ab')2 is 2-4 after reduction. If you have F(ab')2 from a polyclonal or other IgG subtype, you can purchase the Thiol Assay Kit with Purification (Product Number: **CM90005**) separately from CellMosaic to measure the free thiols while letting the reducing solution sit at 4°C in **Step C5**. Use 6 µL F(ab')2

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solution from **Step C5** and follow the protocol of CM90005. To calculate the number of thiols, please use the F(ab')2 concentration of 13 µM.

The assay will take 30 minutes. The number of thiols per F(ab')2 (**n**) is satisfactory within 2-4. If n is lower (i.e., <2.0), you can add additional Reagent A solution from **Step C2** based on the following calculation. Repeat **Step C4,** but mixing at 37°C for 30 minutes will be sufficient, and then cool the F(ab')2 reducing solution to approximately 4°C for 5 minutes before moving to the next purification step.

**Calculation for Additional Reagent A Solution for Targeting Total 4 Thiols per F(ab')2**:

$$Volume\ of\ Additional\ Reagent\ A\ solution\ to\ be\ transferred\ from\ Step\ \boldsymbol{C2}\ (\mu L) = Ab\ in\ mg\ \times 3.42\ \times \left(\frac{4-n}{n}\right)$$

## 5. Purification to Remove Excess Reagent A

The following steps follow a similar filtration process as described in **Steps B1–B11**. These 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 (CM03CD030A), Collection Tubes (CM03CT0), Labeling Buffer (CM02005, indigo label), Clean Centrifuge Tubes (not provided in the kit), F(ab')2 Solution from **Step C5**.

**D1**. Transfer the reduced F(ab')2 solution from **Step C5** directly into a new **Filter Device**. Rinse the centrifuge tube once with 200 µL **Labeling Buffer** and transfer this solution to the **Filter Device.** Add Labeling Buffer to bring the total volume to 500 µL.

**D2**. Spin the **Filter Device** at 14,000 x g for 10 minutes to concentrate the sample to < 100 µL.

**D3**. Transfer the filtrate from the collection tube to a clean centrifuge tube. **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. Spin at 14,000 x g to concentrate for 10 minutes to concentrate to **< 100 µL**. Transfer the filtrate from the collection tube to a clean centrifuge tube. **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 sample tube from **Step D6**.

**D8**. Repeat **Step D7** washing once.

**D9**. Add **Labeling Buffer** to the tube to bring the total volume of the sample to **640 ± 10 µL**.

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**Calculation 3 for Less F(ab')2**:

$$Volume\ of\ Reduced\ F(ab')2\ in\ Step\ \boldsymbol{D9}\ (\mu L) = F(ab')2\ in\ mg\ \times 292$$

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

## 6. MMAE Labeling (Step 2 in Scheme 1)

Items needed: MMAE solution from **step A3**, Hazardous Waste Bag (CM03HZ1), F(ab')2 Solution from **step D10**.

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

**E2**. Transfer the entire solution (**110 µL** total) to the centrifuge tube containing F(ab')2 from **Step D10**. When you add the MMAE solution, place the pipette tip inside the F(ab')2 solution and then dispense the MMAE slowly while swirling the pipette tip. **Dispose of the pipette tip and MMAE tube in the hazardous waste bag**.

**Calculation 4 for Less F(ab')2**:

$$Volume\ of\ MMAE\ Solution\ to\ be\ Transferred\ in\ Step\ \boldsymbol{E2}\ (\mu L) = F(ab')2\ in\ mg\ \times 36.7$$

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

**E3**. 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, Storage Buffer (1x PBS), 2.0 mL Centrifuge Tube (CM03CT3), Hazardous Waste Bag (CM03HZ1), F(ab')2 Solution from **Step E3**.

**F1.** In a chemical hood, securely attach the **Desalting Column** to a support stand, lab frame, or any support rod. Remove the top and bottom caps from the column, allowing the excess liquid to flow through by gravity. Collect the liquid in a flask.

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

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

**F4.** Spin the MMAE-labeled F(ab')2 solution from **Step E3** before opening the tube. Add the entire F(ab')2 solution to the column. **Dispose of the centrifuge tube in the hazardous waste bag.**

**F5.** Add 250 µL of **Storage Buffer** to the column and allow 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 F(ab')2**:

$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F5}\ (\mu L) = 1000 - F(ab')2\ in\ mg\ \times 342$$

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**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 F(ab')2 (Ab)**:

$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F6}\ (\mu L) = 500 + F(ab')2\ in\ mg\ \times 342$$

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

## Conjugate is Ready for Your Experiment

- **Specification for your product:** MMAE-labeled F(ab')2 with an average drug-to-F(ab')2 ratio (DAR) of 2–4. The actual DAR will depend very much on the type of F(ab')2 you are using. A typical batch contains over 95% conjugated products and is free of any unreacted drug. The approximate concentration of the conjugate is 0.88 mg/mL in PBS buffer assuming a 50% recovery. You can determine the concentration and estimate the DAR of the conjugate by UV/vis spectrophotometry (see "Other Considerations").

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## Other Considerations

### 1. Concentration Determination for F(ab')2 (Unlabeled)

Accurately determining the F(ab')2 concentration is crucial for obtaining an optimized DAR in this protocol. The simplest method for measuring F(ab')2 concentration in solution is to measure the absorbance at 280 nm (UV range) ($A_{1\ mg/mL}$ = 1.4).

If your F(ab')2 is in a buffer that does not absorb at 280 nm, you can measure the UV absorbance directly prior to starting an experiment.

$$Concentration\ (mg/mL)\ of\ F(ab')2 = \frac{(A280)}{1.4}$$

If your F(ab')2 is in a buffer that absorbs at 280 nm, determine the concentration in **step B11** after exchanging with Reducing Buffer, assuming **95%** recovery of the F(ab')2. Reducing Buffer does not interfere with the UV measurement at 280 nm. The total volume of Reducing Buffer added in **Step B10** can be estimated based on the initially estimated amount of F(ab')2 and will not affect the conjugation too much if the volume is off to some extent.

$$Concentration\ (mg/mL)\ of\ Starting\ F(ab')2 = \frac{(A280)}{1.4 \times 0.95}$$

After calculating the total amount of F(ab')2, follow the calculations in **Steps B10, C3**, **D10**, **E3**, **F5,** and **F6** to ensure correct volumes are used in each step.

### 2. Concentration Determination for Conjugate

To determine the concentration of the conjugate, 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 formula:

$$Concentration\ (\mu M) of\ the\ dilute\ sample = \frac{(A280) \times 6.4935}{L}$$

$$Concentration\ (mg/mL) of\ the\ dilute\ sample = \frac{(A280) \times 0.974}{L}$$

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 F(ab')2 with MW of 110,000, the molar extinction coefficient is 154,000 $M^{-1}cm^{-1}$.

### 3. MW Calculation

Calculation of the MW of the conjugate:

**MW(Conjugate) = n x 1317+ 110000**

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Where *n* is the average molar ratio of MMAE per F(ab')2. Use a value of 2.0 if you do not have the experiment data.

## 4. Drug-to-F(ab')2 Ratio (DAR) and Characterization by UV and HPLC

In this kit, the target DAR is 2-4. The actual DAR will depend very much on the type of F(ab')2 you have.

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.

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

Then, you can also use the following formula to estimate the DAR (for reference only):

$$DAR = \frac{(15.4 \times R - 6.6)}{(1.615 - 0.1425 \times R)}$$

**Note:** The UV contribution of the VC-PAB-MMAE to the conjugate is experimentally determined at CellMosaic. The UV absorbance of the VC-PAB-MMAE in an conjugate can vary significantly due to factors like aggregation and stacking. Therefore, the ***R*** value for an conjugate may differ greatly depending on the F(ab')2 and should be determined experimentally. The DAR calculation using this formula is for reference purposes 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 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 aggregation during the reaction. The precipitate will be removed during purification. Depending on the properties of your F(ab')2, recovery will be 40-80%. If you are

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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 IgG and the ADC, you can estimate how much aggregation is in the ADC.

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.

## 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.

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

**F(ab')2 information**: F(ab')2 was prepared using CellMosaic's PerKit™ F(ab')2 preparation kit (Cat#: CM51408) from a mouse IgG1 F(ab')2.

**Kit Lot number**: 5525.S13.032919

**Scale of the reaction**: 0.5 mg

**DAR of the final conjugates**: 2 (Calculated based on the UV absorbance ratio (R) of the conjugate at 248 nm and 280 nm)

**Figure 1**: HIC HPLC analysis of F(ab')2 and purified conjugates

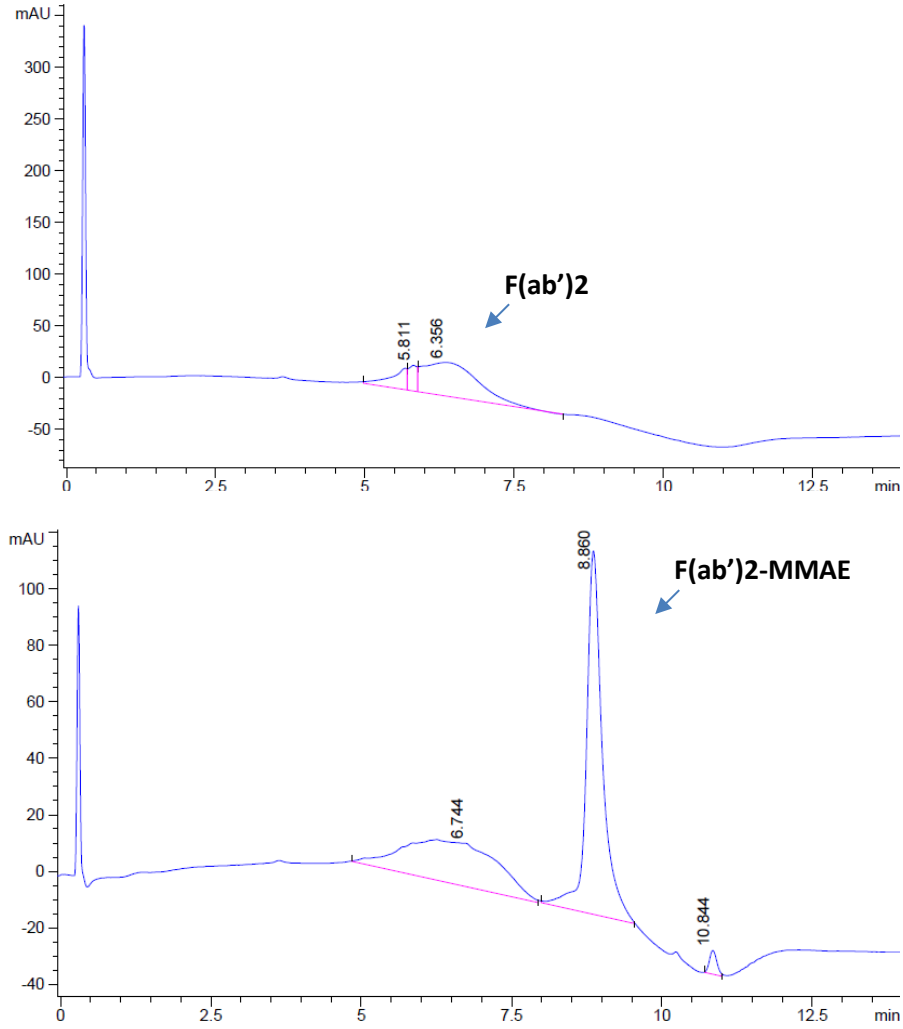


**Figure 2**: SEC HPLC analysis of F(ab')2 and purified conjugates

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