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PerKit™ Antibody or Protein PBD Dimer Conjugation Kit with Val-Ala Linker (CM11439x1 and CM11439x3) User Reference Guide

Contents

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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.

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Kit Components

Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are a class of antitumor antibiotics that were discovered in the 1960s. The first PBD, anthramycin, was isolated from the cultures of *Streptomyces*. PBDs are sequence-selective DNA minor-groove binding agents that inhibit nucleic acid synthesis. PBD dimers, with two PBD units linked via their C8 positions, are more potent DNA alkylating agents than monomers and have been used by several companies to develop antibody-drug conjugates. Examples include Rovalpituzumab tesirine (AbbVie/Stemcentrx), Vadastuximab talirine (Seattle Genetics), Camidanlumab tesirine, and Loncastuximab tesirine (ADC Therapeutics).

This kit provides materials to conjugate 6.67 to 20 nmol of an antibody or protein sample (CM11439x1) or up to three separate antibody or protein samples (CM11439x3), each ≥20 kDa, with the talirine (SGD-1910) PBD dimer using a valine-alanine 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 (CM11413x1)	Quantity (CM11413x3)	Storage condition
Box 1	Mc-Val-Ala-PBD Dimer (**red label**)	CM11030.1	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	40 mL	
	Storage Buffer (1 x PBS buffer) (**grey label**)	CM02013	20 mL	60 mL	
	ADC Stabilizing PBS buffer (5x) (**pink label**)	CM02022	0.5 mL	1.5 mL	
	Centrifugal Filter Devices	CM03CD010A	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	Protein (MW: ≥20 kDa)	N/A	NOT PROVIDED (User Supplied Material): Antibody or protein: **6.67-20 nmol** per reaction IgG (MW of 150kDa): **1-3 mg** per reaction		

Reaction Scale: The protocol is optimized for conjugating 20 nmol of antibody or protein. If you have less than 20 nmol of antibody or protein, use the calculations in **Steps B10**, **C3**, **D9**, **E2, E5, F5, F6,** and **F7** to determine the correct volumes to add at each step.

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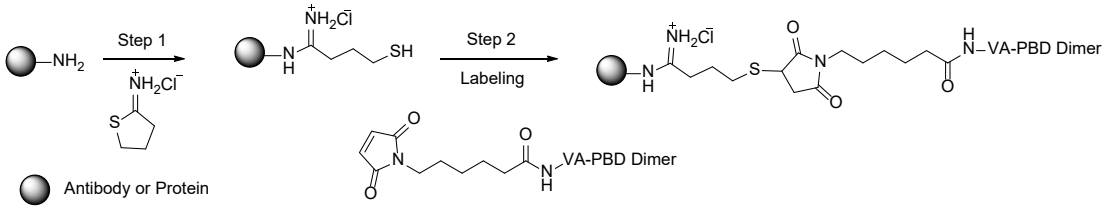
Drug-to-Antibody or Protein Ratio (DAR or DPR): The protocol is optimized for IgG (150 kDa) to achieve an average DPR of 2–4. For other antibodies or proteins, the DAR or DPR may vary from 1–4, depending on the molecular weight of the antibody or protein.

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 antibody or protein with PBD dimer using a dipeptide (Val-Ala) linker. The user supplies the antibody or protein. Using the kit components, the user first converts the antibody or protein into a thiol-protein through surface amine labeling, followed by reaction with maleimide-activated PBD dimer to generate antibody- or protein-PBD dimer conjugates. The product is then purified to remove any unreacted drug.



Key features of this conjugation kit:

- Simple and efficient labeling of antibody or protein with PBD dimer, minimizing toxin exposure.
- Features an enzymatic dipeptide (Val-Ala) linker.
- Fast preparation: 6 hours total, with less than 2 hours of hands-on time.
- Includes all necessary reagents and supplies for preparation and purification.
- Post-conjugation services available at CellMosaic® for analysis and drug loading determination.

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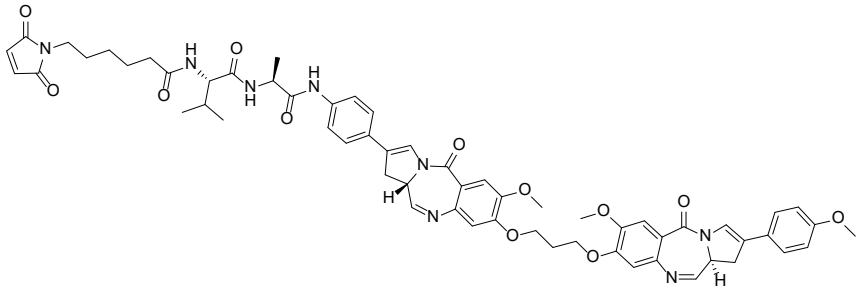
Drug Information:



- **Name**: Mc-Val-Ala-PBD Dimer (Talirine, SGD-1910)
- **CAS number**: 1342820-51-2
- **Chemical formula**: $C_{60}H_{64}N_8O_{12}$
- **MW**: 1089.2
- **Mechanism of action**: Sequence-selective DNA minior-groove binding agents, inhibition of nucleic acid synthesis
- **Activities**: Antitumor

Requirement for antibody or protein:
1. Preferably > 90% pure by gel electrophoresis
2. Total amount: 6.67-20 nmol antibody or protein content as measured by UV. *Note*: the accuracy of your antibody or protein measurement is the single most important factor in obtaining an optimized DAR or DPR of 2–4. Please refer to the "Other Considerations" section in this manual for instructions on measuring the antibody or protein amount.

Support

A customer can request a recommendation for conjugation if their antibody or protein has special features or if they need to label less than 6.67 nmol or a protein with a MW below 20kDa.

CellMosaic provides additional accessory tools, such as buffers, standards, and reagents for antibody-drug conjugate (ADC) or protein-drug conjugate (PDC) research. The company also offers fee-based support services to customers who need assistance with final conjugate analysis by HPLC and drug loading determination.

If an ADC or PDC cannot be synthesized using classical linkers due to drug hydrophobicity, or if ADC efficacy is compromised by drug loading, customers can explore CellMosaic's proprietary hydrophilic and water-soluble AqT® Linked Drugs for the development of next generation ADCs and PDCs.

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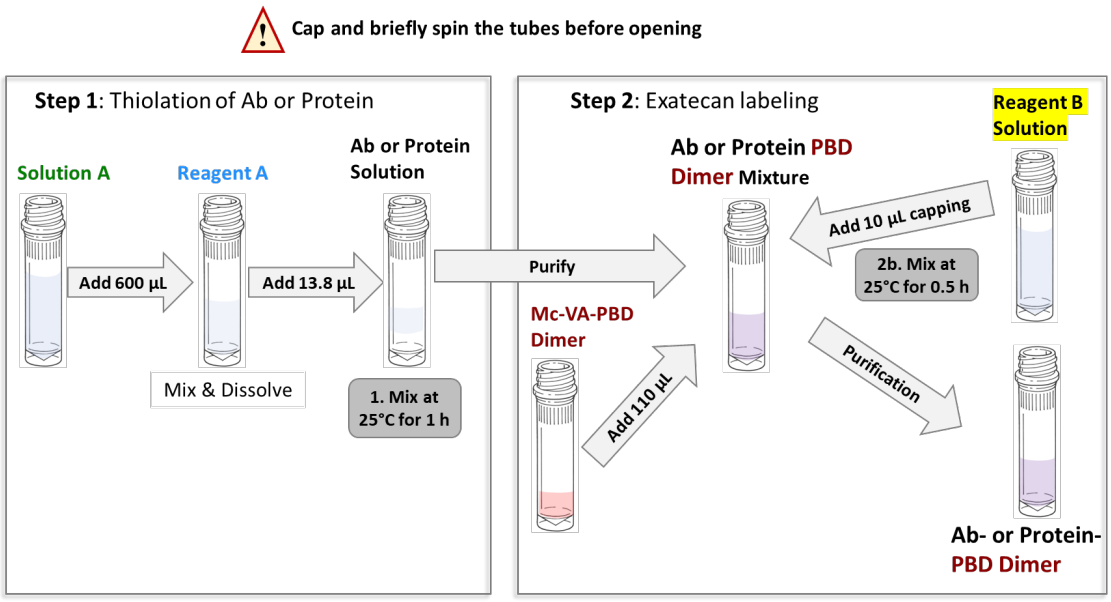
Protocol



Scheme 1. Schematic workflow diagram for preparing Antibody- or Protein-PBD dimer conjugates with Val-Ala linker, starting with 20 nmol of antibody or protein (Reagent volume will vary if the amount of antibody or protein is less than 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 and at RT
- Chemical hood
- Support stand, lab frame, or any support rod for desalting column
- Flask
- Personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves)

2. Prepare Site and PBD Dimer for Labeling Experiment

PBD dimer is highly hydrophobic, and antibody- or protein-drug conjugates with PBD dimer tend to aggregate and precipitate out of solution. Therefore, it is recommended to perform the labeling experiment just a few days before your subsequent experiments.

Always use personal protection equipment when handling PBD dimer. Ensure you are working in a clean space inside a chemical fume hood.

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A1. Remove **Box 1** containing **PBD dimer** (red label), **Reagent A** (cyan label), and **Reagent B** solution (yellow 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 **PBD dimer** tube has thawed. Briefly mix and spin the centrifuge tube containing PBD dimer. Place the PBD dimer tube in a tube holder inside the chemical hood and wait until the antibody or protein 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 incubator or shaker temperature to 25°C.

3. Preparation of Antibody or 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).

The total amount of antibody or protein used for the conjugation is 20 nmol per reaction. The protocol is optimized for the IgG antibody with a molecule weight (MW) of 150 kDa to obtain an average of 2–4 drugs per IgG.

Calculation: Amount of antibody or protein (mg) = MW of antibody or protein x 0.00002

Reaction Scale: If you have less than 20 nmol of antibody or protein, refer to the calculations in **Steps B10**, **C3**, **D9**, **E2, E5, F5, F6,** and **F7** to determine the correct volumes to add at each step.

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 or protein.

- ✓ **Lyophilized antibody or protein**: Dissolve the antibody or protein in 500 µL of deionized water and transfer the entire contents to the Filter Device.
- ✓ **Antibody or protein in < 500 µL buffer**: Transfer the antibody or protein sample directly to the Filter Device, then add Buffer A to bring the total volume to 500 µL. Cap the device.
- ✓ **Antibody or protein in 500-1000 µL buffer**: Split the sample between two Filter Devices, adding the antibody or protein to each device. Add Buffer A to bring the volume in each device to 500 µL and cap them.
- ✓ **Antibody or protein 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 or

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protein sample has been transferred. For the final refill (**Step B5),** add Buffer A 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 10 minutes (preferably cooled at 4°C) to concentrate the sample to **< 100 µL** (Spin time may vary; typically, a 500 µL sample will concentrate to ~50µL after 10 to 20 minutes of spinning. The typical time for an Eppendorf 5417R is 10 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 Buffer A 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 20-80 µL of Buffer A to the Filter Device for rinsing (the actual volume of Buffer A will depend on the total volume calculated in **Step B10**). Stir gently with a pipet tip, then transfer the contents to the sample tube from **Step B7.**

B9. Repeat **Step B8** washing once.

B10. Add Buffer A to the sample tube from **Step B9** to bring the total volume of the sample to **290 ± 5 µL.** Cap the tube.

Calculation 1 for Less Antibody or protein:

$$Total\ volume\ of\ the\ sample\ in\ Step\ \boldsymbol{B}\mathbf{10}\ (\mu L) = Antibody\ or\ Protein\ in\ nmol\ \times 14.5$$

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

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

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

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

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

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C3. Transfer Reagent A solution from **Step C2** to the centrifuge tube containing the antibody or protein from **Step B11.** Discard any unused Reagent A as hazardous chemical waste **after completing all experiments**.

The volume of Reagent A solution is adjusted based on the molecular weight (MW) of your antibody or protein to ensure optimal thiol groups loading for ADC or PDC preparation, minimizing aggregation. Use the following guide:

MW of Antibody or Protein	Volume of Reagent A (µL)	Target PBD Dimer per Antibody or Protein
⩾100 kDa	13.8 µL	2 – 4
60–100 kDa	10 µL	1–3
20–60 kDa	8 µL	1–3

Calculation 2 for Less Antibody or Protein:

MW (≥100kDa):

$$Volume\ of\ Reagent\ A\ solution\ to\ be\ transferred\ in\ Step\ \boldsymbol{C3}\ (\mu L) = Antibody\ or\ Protein\ in\ nmol\ \times 0.69$$

MW (60-100kDa):

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

MW (20-60kDa):

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

C4. Vortex the solution for 30 seconds, then spin briefly to ensure no liquid remains in the cap. Incubate the mixture at 25°C (room temperature, 20–27°C is acceptable) for exactly 1 hour.

Start Time: _____ End Time:_____

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 mix it by pipetting every 10 minutes.

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 free thiols oxidize quickly. Ensure that **Step A3** is completed before proceeding. Work quickly through **Steps D6–D10.**

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Items needed: Filter Device (CM03CD010A), Collection Tubes (CM03CT0), Buffer B (CM02005, indigo label), Mc-Val-Ala-PBD Dimer (CM11030.1, red label), Reagent B Solution (CM12004.1, yellow label), 1.5 mL Centrifuge Tube (CM03CT2), Antibody or Protein Solution from **Step C4**.

D1. Transfer the thiol-modified antibody or protein from **Step C4** directly into a new Filter Device. Rinse the centrifuge tube once with 200 µL of Buffer B (indigo label) and transfer this solution to the Filter Device. Add Buffer B 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 Buffer B to bring the total volume to 500 µL. Spin at 14,000 x g for 10 minutes to concentrate to < 100 µL. Transfer the filtrate to a clean centrifuge tube. **Save the filtrate until all experiments are done.**

D5. Repeat **Step D4** once.

Work quickly

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.

D7. Add 50-200 µL of Buffer B to the Filter Device for rinsing (the exact volume of Buffer B 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 Buffer B to the sample tube to bring the total volume of the sample to **640 ± 10 µL**.

Calculation 3 for Less Antibody or Protein:

$$Total\ volume\ of\ the\ sample\ in\ Step\ \boldsymbol{D9}\ (\mu L) = Antibody\ or\ Protein\ in\ nmol\ \times 32$$

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

6. PBD Dimer Labeling (Step 2 in Scheme 1)

Items needed: PBD Dimer Solution from **step A3**, Hazardous Waste Bag (CM03HZ1), Antibody or Protein Solution from **step D10**.

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

E2. Transfer the entire solution (**110 µL** total) to the tube containing the antibody or protein from **Step D10**. When adding the PBD dimer solution, insert the pipette tip into the antibody or protein solution and slowly dispense the PBD dimer while gently swirling the pipette tip.

Dispose of the pipette tip and PBD dimer tube in the hazardous waste bag.

Calculation 4 for Less Antibody or Protein:

$$Volume\ of\ Exatecan\ Solution\ to\ be\ Transferred\ in\ Step\ \boldsymbol{E2}\ (\mu L) = Antibody\ or\ Protein\ in\ nmol\ \times 5.5$$

Dispose any unused PBD dimer solution as hazardous chemical waste.

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E3. Cap the tube and mix the solution at 25°C (room temperature, 20–27°C is acceptable) for 1 hour.

Start Time: _____ End Time:_____

E4. Spin the centrifuge tube containing Reagent B solution (Yellow label).

E5. Spin the PBD dimer-labeled antibody or protein solution from **Step E3** before opening it. Add **10 µL** of Reagent B solution from **Step E4** to the tube. **Discard any unused Reagent B as hazardous chemical waste after completing all experiments**.

Calculation 5 for Less Antibody or Protein:

$$Volume\ of\ Reagent\ B\ solution\ to\ be\ Transferred\ in\ Step\ \boldsymbol{E5}\ (\mu L) = Antibody\ or\ Protein\ in\ nmol\ \times 0.5$$

E6. Vortex the solution for 30 seconds, then spin it down. Mix the solution at 25°C (room temperature, 20–27°C is acceptable) for 30 minutes.

Start Time: _____ End Time:_____

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 Column (CM03SG10), Storage Buffer (1x PBS), 2.0 mL Centrifuge Tube (CM03CT3), ADC Stabilizing PBS Buffer (5x) (CM02022, pink label), Hazardous Waste Bag (CM03HZ1), Antibody or Protein 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 PBD dimer-labeled antibody or protein solution from **Step E6** before opening the tube. Add the entire antibody or protein solution to the column. **Dispose of the centrifuge tube in the hazardous waste bag.**

F5. Add 250 µL of Storage Buffer to the column**,** allowing the liquid to fully enter the gel bed. (***Note:*** This elution buffer does not contain any of your products and can be discarded as waste).

Calculation 6 for Less Antibody or Protein:

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$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F5}\ (\mu L) = 1000 - Antibody\ or\ Protein\ in\ nmol\ \times 37.5$$

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 7 for Less Antibody or Protein:

$$Volume\ of\ Storage\ buffer\ in\ Step\ \boldsymbol{F6}\ (\mu L) = 500 + Antibody\ or\ Protein\ in\ nmol\ \times 37.5$$

F7. Add ADC Stabilizing PBS buffer (5x) to the antibody or protein-PBD Dimer from **Step F6**. Aliquot and store the conjugate in a < -20°C freezer or lyophilize to dryness. **Dispose of the Desalting Column in the hazardous waste bag and seal the bag. Dispose of the waste following regulations appropriate for your area.**

Calculation 8 for ADC Stabilizing PBS Buffer (5x) Added:

$$Volume\ of\ Stabilizing\ Buffer\ (\mu L) = 0.25\ \times Volume\ of\ the\ Conjugate$$

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

Conjugate is Ready for Your Experiment

- **Specifications of your product:** A typical batch contains around 70–90% conjugated products, as determined by hydrophobic interaction chromatography (HIC) with an average of 1–4 drugs per antibody or protein and is free of any unreacted drug. The approximate concentration of the conjugate is 6.4 µM (0.96 mg/mL for IgG) assuming 50% recovery. You can determine the concentration and estimated the loading by UV/vis spectrophotometry (see Other Considerations).

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Other Considerations

1. Concentration Determination for Antibody or Protein (Unlabeled)

Accurately determining the antibody or protein concentration is crucial for optimizing drug loading in this protocol. The simplest method is to measure absorbance at 280 nm, provided you know the extinction coefficient of your antibody or protein. Otherwise, an antibody or protein assay, such as BCA, can be used to determine the concentration.

If your antibody or protein is in a buffer that does not absorb at 280 nm, you can measure the UV absorbance directly before starting the experiment.

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

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

If your antibody or protein is in a buffer that absorbs at 280 nm, determine the concentration after buffer exchange with Buffer B (**step B10)**, assuming **95%** recovery of the antibody or protein. Buffer A does not interfere with UV measurement at 280 nm. The total volume of **Buffer A** added in **Step B10** is estimated based on the initially calculated amount of **antibody or protein**. Minor variations in volume will not significantly affect the conjugation process.

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

After calculating the total amount of antibody or protein, follow the calculations in **Steps B10**, **C3**, **D9**, **E2, E5, F5, F6,** and **F7** to ensure correct volumes are used in each step.

2. Concentration Determination for Conjugate

To determine the conjugate concentration, dilute your sample from **Step F7** with 1x PBS buffer.

Dilute ADC Stabilizing PBS buffer 5 times with 1xPBS and then dilute this PBS buffer in the same way as the conjugate to serve as a buffer control.

Measure the UV absorbance of the conjugate at 280 nm (A280) using a UV spectrometer and calculate the conjugate concentration using the following formula:

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

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

Where **L** is the UV cell path length (cm), and **ε** is the extinction coefficient of your antibody or protein ($cm^{-1}M^{-1}$). If you are using a 1 cm UV cell, you may dilute the conjugate 2-4 times to obtain an accurate reading.

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The contribution from the drugs at 280 nm is considered very small and is not taken into account here.

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

3. MW Calculation for Conjugate

Calculation of the MW of the conjugate:

MW(PDC) = *n* x 1227+ MW (Antibody or Protein)

Where *n* is the average molar ratio of PBD dimer per antibody or protein. If the experimental DPR is unavailable, use 1.5 for antibody or protein with MW of 20-60kDa, 2 for antibody or protein with MW 60-100 kDa, 3 for antibody or protein with MW ≥100 kDa.

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

PBD dimers have a characteristic UV absorbance maximum at around 330 nm. The presence of a UV peak at 330 nm indicates drug loading. For drug loading determination, we strongly recommend sending your samples for intact mass spectrometry (MS) analysis using either MALDI-TOF MS or LC-MS. By comparing the intact MS spectra of the conjugate with those of the unlabeled antibody or protein, you can calculate the average drug-to-antibody ratio (DAR) or drug-to-protein ratio (DPR). If you do not have access to an MS facility, please contact CellMosaic for analysis.

5. Characterization of Conjugates by HIC HPLC

For conjugate prepared via surface amine labeling of the antibody or protein, hydrophobic interaction chromatography (HIC) HPLC can be used to determine whether labeling has occurred. However, due to the highly heterogeneous nature of surface amine labeling, antibodies or proteins with the same DAR or DPR may exhibit slight differences in hydrophobicity. For a typical HIC profile of PBD dimer conjugate, please refer to 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 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 Conjugates by SEC HPLC

PBD dimer with Val-Ala is highly hydrophobic. This kit is designed to minimize the aggregation and precipitation issues typically encountered with PBD dimer labeling. However, you may still notice some solid precipitate or conjugate aggregation during the reaction. The precipitates will be removed during purification. Depending on the properties of your antibody or protein, recovery may range from 40-80%.

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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 conjugates elute earlier. By comparing the SEC profile of unlabeled antibody or protein to that of the conjugate, you can estimate the level of aggregation in the conjugate.

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 conjugate precipitation. The Stabilizing Buffer also helps preserve the structure of the conjugate 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

This antibody- or protein-PBD dimer conjugate with a traditional hydrophobic linker is not very stable due to aggregation and precipitation issues. The stability of your conjugate may vary depending on your antibody or protein and should be assessed using HPLC or UV analysis. If you need to store the conjugates for a longer period, dilute your conjugate 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) 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 conjugate (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: Examples of Antibody- or Protein-PBD Dimer Conjugates

Example: Mc-Val-Ala-PBD Dimer Conjugation with Monoclonal Human IgG1 Subtype Antibody

The ADC was prepared at CellMosaic following the User Manual (Rev. A) with some adjustments.
Mc-Val-Ala-PBD Dimer lot number(s): S576.S7.1126D
Scale of the reaction: 6.67 nmol (1.0 mg) Antibody

Specifications of the final conjugates:
Calculated DAR based on the HIC data: ~2.7 (Multiple DAR products)
Unreacted antibodies: 21.3%
ADC recovery: 75%
Aggregation: 12.3%

Figure 1: HIC HPLC analysis of monoclonal human IgG1 (Panel A), and purified Antibody-Val-Ala-PBD Dimer conjugates (Panel B).

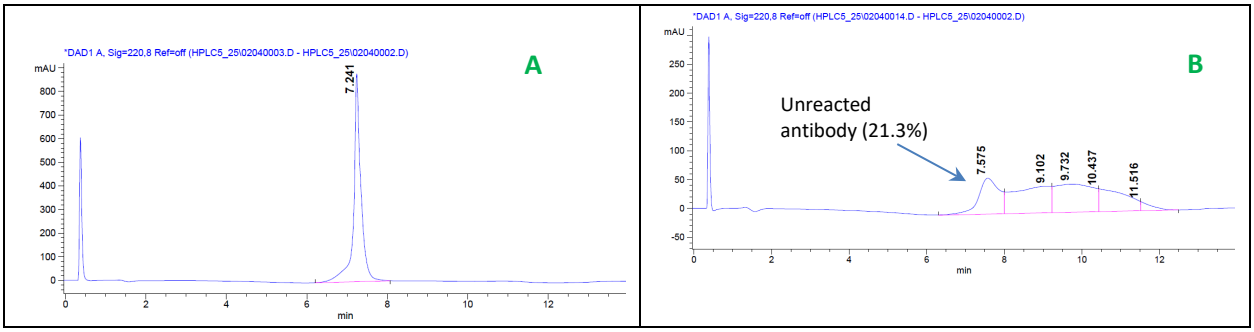

Figure 2: SEC HPLC analysis of monoclonal human IgG1 (Panel C), and purified Antibody-Val-Ala-PBD Dimer conjugates (Panel D) at 280 and 330 nm. 330 nm absorbance is characteristic of PBD dimer.