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PerKit™ Antibody Peptide Conjugation Kit (CM32402x1 and CM32402x3) User Reference Guide

Contents

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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 a Cys-peptide onto one (CM32402x1) or three (CM32402x3) antibody samples (IgG). Scale of each reaction: 3 mg (protein content).

⚠ 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	Cat#	Quantity (CM32402x1)	Quantity (CM32402x3)	Storage Condition
Box 1	EMCS (red label)	CM12107.1	1 unit	3 unit	-20°C
Box 2	Solution A (blue label)	CM01008	0.5 mL	0.5 mL	2-8°C
	Solution B (green label)	CM01003	1 mL	1 mL	
	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	4 mL	12 mL	
	Filter Devices for Antibody	CM03CD50A	3	9	
	Filter Devices for Conjugate	CM03CD100A	2	6	
	Collection Tubes	CM03CT0	10	30	
	1.5 mL Centrifuge Tubes	CM03CT2	3	9	
	0.5 mL Centrifuge Tube(s)	CM03CT1	1	3	
User Supplied Material	IgG Antibody	NOT PROVIDED (User supplied material, 3 mg antibody per reaction)			
	Cys-peptide	NOT PROVIDED (User supplied material, 0.6 µmol per reaction)			

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 IgG antibody with any peptide containing a reactive Cys group using EMCS crosslinker. The user supplies the antibody and peptide. The kit includes EMCS, which can be coupled first to the antibody to introduce the maleimide groups on the surface, and then the maleimide groups react with the Cys of the peptide to form the conjugates. The product is purified to remove any unreacted peptides.

Key features of this conjugation kit:

- Offers a simple and easy way to label IgG with peptide

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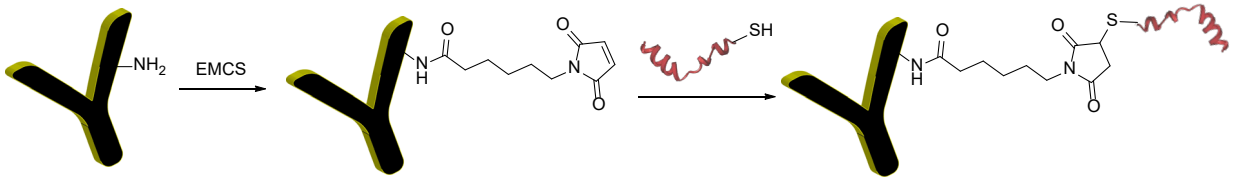
- Fast and easy preparation: 4 h preparation and <2 h hands-on time
- Peptide loading is adjustable
- All reagents and supplies included for preparation and purification
- >95% conjugated products (free of or <5% unreacted peptide)



Requirement for antibody (IgG):
1. Preferably >90% pure by gel electrophoresis
2. Total amount: 3 mg (protein content)

Requirement for Cys-peptide:
1. Amount: 0.6 µmol
2. HPLC purified and lyophilized: please ensure no reducing reagents, such as DTT, are present
3. The Cys-peptide should be stored at -80°C
4. HPLC purity: >85% for *N*-terminal Cys-peptide and >90% for *C*-terminal Cys-peptide

Potential interfering compounds for labeling and conjugation reactions:
Thiols: e.g., DTT and mercaptoethanol

Support

Customer can request a recommendation for the conjugation if the peptide has a special feature or a low amount of antibody. CellMosaic also provides fee-based support services to customers who need help analyzing the final conjugates by HPLC and determining the loading by mass.

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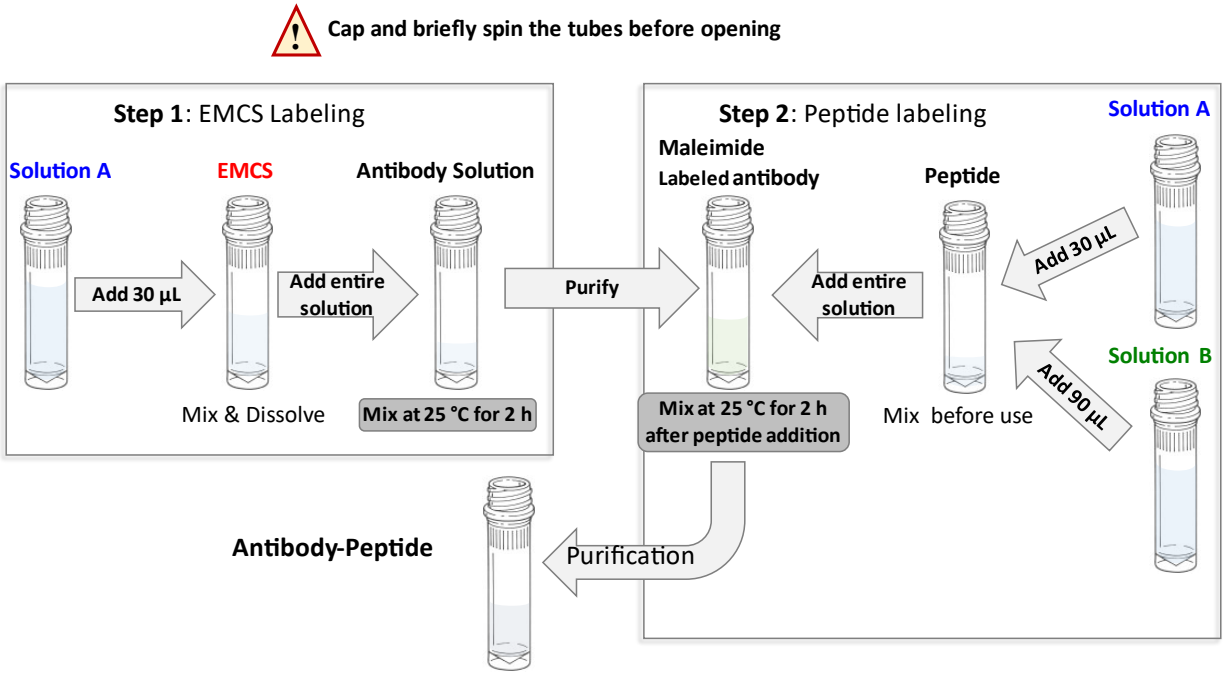
Protocol



Scheme 1. Schematic diagram of the workflow for preparing antibody–peptide conjugates

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 g capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C
- 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. Preparation of Antibody Samples for Conjugation

Items needed: IgG Antibody (user supplied), Filter Devices for Antibody (CM03CD50A), 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 antibody used for the conjugation is 3 mg (protein content measured by UV).

A1. Insert one **Filter Device for Antibody** into one of the provided **Collection Tubes** (microcentrifuge tube with the cap attached). Perform the step based on the following conditions.

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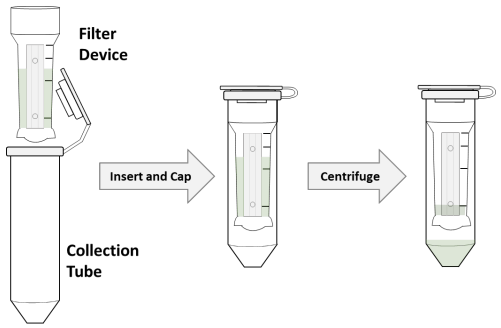
- ✓ If your antibody is supplied as a lyophilized solid, dissolve the antibody in 500 µL of **deionized water** and then transfer the entire contents to the **Filter Device**.
- ✓ If your antibody is supplied in < 500 µL buffer, transfer your antibody sample to the **Filter Device** directly. Add **Buffer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



- ✓ If the volume of your antibody sample is between 500 and 1000 µL, divide the volume into two **Centrifugal Filter Devices** and add the antibody 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 antibody sample is >1000 µL, add up to 500 µL of sample to the two **Filter Devices** and cap them. Repeat Steps **A1-A4** until all of the antibody sample has been added the **Filter Device**. Move on to Step **A5**. Add **Buffer A** to make up the total volume to 500 µL in each device for the last refill.

A2. Place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

A3. Spin the **Filter Device** at 14,000 x g for 8 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 8 to 20 minutes. The typical volume is ~40 µL after spinning for 8 minutes on an Eppendorf 5417R at 4°C).

A4. 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.**

A5. 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. 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.**

A6. Repeat **Step A5** two more times.

A7. Transfer the concentrated sample from the **Filter Device** to a **1.5 mL Centrifuge Tube** (use a pipetman to measure the approximate volume of the concentrated sample).

A8. Add 100 µL of **Buffer A** to the **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step A7.**

A9. Repeat **Step A8** once.

A10. Add **Buffer A** to the 1.5 mL micro-centrifuge tube from **Step A9** to make up the total volume of the sample to **570 ± 5 µL** and cap it.

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A11. Vortex the combined antibody sample for 30 seconds and then centrifuge to ensure no liquid is in the cap.

3. EMCS Labeling (Step 1 in Scheme 1)

Items needed: Antibody sample from Step A11, Buffer A (CM02001, orange label), Solution A (CM01008, blue label), EMCS (CM12107.1, red label).

B1. Spin the centrifuge tube containing **EMCS** (red label) before opening it.

B2. Spin **Solution A** (blue label) to ensure there is no liquid in the cap before opening it. Add **30 µL** of **Solution A** to the **EMCS** tube from **Step B1**. Vortex for 30 seconds to 1 minute to dissolve the reagent and then centrifuge to ensure no liquid is in the cap.

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

B3. Transfer **EMCS solution** from **Step B2** to the antibody solution from **Step A11**. The volume of EMCS solution being transferred will depend on the target loading:

High peptide loading (8-15 maleimides per antibody): **30 µL** of EMCS solution

Medium peptide loading (4-8 maleimides per antibody): **16 µL** of EMCS solution

Low peptide loading (2-4 maleimides per antibody): **8 µL** of EMCS solution

Note: The above targeted loading is an estimate from the labeling done at CellMosaic. The actual number of maleimide groups loaded onto the antibody may vary for individual antibody (e.g. the number of accessible surface amines will affect the loading). The conjugation between maleimide antibody and Cys-peptide labeling is usually very effective (near quantitative).

B4. Vortex the reaction mixture from **Step B3** for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix at 25°C or RT for 2 h.

Start Time: _____ End Time:_____

Tip for mixing: You can use a nutator, shaker, vortex, or 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.

4. Purification to Remove Excess EMCS

Items needed: Antibody sample from Step B4, Buffer B (CM02005, indigo label), Filter Device for Antibody (CM03CD50A), Collection Tubes (CM03CT0), 1.5 mL Centrifuge Tube (CM03CT2), clean centrifuge tubes (not provided in the kit).

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C1. Insert the **Filter Device for Antibody** into one of the provided collection tubes (microcentrifuge tube with the cap attached). Transfer 500 µL of the EMCS-labeled antibody solution from **Step B4** into the **Filter Device** directly. Place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

C2. Spin the **Filter Device** at 14,000 x g for 8 minutes (preferably cooled to 4°C) to concentrate to < 100 µL.

C3. 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.**

C4. Insert the **Filter Device** back into the collection tube. Transfer the rest of the EMCS-labeled antibody solution from **Step B4** into the **Filter Device** directly. 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.**

C5. Insert the **Filter Device** back into 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.**

C6. Repeat **Step C5** two more times.

C7. Transfer the concentrated sample from the **Filter Device** to a **1.5 mL Centrifuge Tube** (use a pipetman to measure the approximate volume of the concentrated sample).

C8. Add 100 µL of **Buffer B** to the **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step C7.**

C9. Repeat **Step C8** once.

C10. Add **Buffer B** to make up the total volume of the sample to **380 ± 5 µL** and cap it.

C11. Vortex the antibody sample from **Step C10** for 30 seconds and then centrifuge to ensure no liquid is in the cap.

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 3.5 µL of the antibody solution from **Step C10** with 66.5 µL of **Buffer A** (included in the maleimide assay kit) for the assay following the protocol provided with the maleimide assay kit. The final concentration of the protein is 52.6 µM if the total volume of the protein is 380 µL (prior dilution).

5. Peptide Labeling (Step 2 in Scheme 1)

Items needed: Antibody sample from Step C11, Solution A (CM01008, blue label), Solution B (CM01003, green label), 0.5 mL Centrifuge Tube (CM03CT1).

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D1. Weigh 0.6 µmol of **Cys-peptide** into a **0.5 mL Centrifuge Tube**. Note, the peptide is statically charged. Use the tip of a glass Pasteur pipet to weigh the peptide if possible. It may be difficult to obtain the exact weight. Any amount between 0.5 µmol and 0.7 µmol is acceptable (no need to adjust the volume of the dissolving solution).

Calculation for 0.6 µmol:

Amount of Cys-peptide ----------(mg) = Molecular weight of Cys-peptide --------- x 0.0006

D2. Add 30 µL of **Solution A** (blue label) to the Cys-peptide tube from **Step D1.** Vortex for 30 seconds and centrifuge the tube to get all the liquid to the bottom. Open the cap and add 90 µL of **Solution B** (green label) to the tube. Vortex for 30 seconds or sonicate for a few minutes to ensure all the solid is dissolved. Discard any unused **Solution A** as hazardous chemical waste **until the experiments are done**.

Tip for solubility check (Step D2): It may take a while for your peptide to fully dissolve. In general, most of the peptide should be able to dissolve in this mixed solution system. Check the bottom of the micro-centrifuge tube to ensure the solution is clear and free of any solid residue. If after a few minutes some solid remains, centrifuge the tube and pipette the supernatant for the next step.
Tip for opening centrifuge tube after vortex: Always centrifuge the tube to ensure no liquid is in the cap.

D3. Transfer the entire solution from **Step D2** to the tube containing **Activated Antibody** from **Step C11**. Pipette the solution up and down in the tube three times to mix. Incubate at room temperature for 2 hours.

Start Time: _____ End Time:_____

6. Purification to Remove Excess Peptide

Items needed: Antibody sample from Step D3, PBS Buffer (CM02013, grey label), 2 Filter Devices for Conjugate (CM03CD100A), Collection Tubes (CM03CT0), 1.5 mL Centrifuge Tube (CM03CT2), clean centrifuge tubes (not provided in the kit).

E1. Place the centrifuge tube containing the reaction mixture from **Step D3** into the centrifuge rotor and counterbalance with a similar device. Spin the centrifuge tube at 10,000 x g for 2 minutes.

E2. Transfer and divide the supernatant from **Step E1** into two **Filter Devices for Conjugate**. Add 250 µL of **PBS Buffer** to make up the total volume to 500 µL in each **Filter Device** and cap them.

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E3. Place the capped **Filter Devices** into the centrifuge rotor, aligning the cap straps toward the center of the rotor. Spin the devices at 14,000 x g for 8 minutes to concentrate to **< 100 µL**.

E4. 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.**

E5. Insert the **Filter Device** back into the collection tube. Add 400-450 µL **PBS Buffer** to make up the total volume to 500 µL. Spin the device at 14,000 x g for 8 minutes 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.**

E6. Repeat **Step E5** two times.

E7. To recover the conjugates, transfer the concentrated sample from the two **Filter Devices** to a **1.5 mL Centrifuge Tube** (use a pipetman to measure the approximate volume of the concentrated sample).

E8. Add 50 µL of **PBS buffer** to each **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step E7.**

E9. Repeat **Step E8** once. Add **PBS Buffer** to make the total volume of the sample 400 µL and cap it.

E10. Vortex the combined protein sample for 30 seconds and then centrifuge to ensure no liquid is in the cap.

Antibody-Peptide is Ready for Your Experiment

Tip: The approximate concentration of the antibody-peptide conjugate is 6 mg/mL PBS buffer (assuming 80% recovery). You can also determine the concentration using a UV/Vis spectrophotometer.

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Other Considerations

1. Concentration Determination

To determine the concentration, dilute your conjugate from **Step E10** 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:

$$Concentration\ (\mu M)\ of\ the\ dilute\ sample = \frac{(A280) \times 4.7619}{L}$$

$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280) \times 0.7143}{L}$$

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

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

2. MW Calculation

Calculation of the MW of the conjugate:

MW(conjugate) = n x MW of peptide+ 150,000

Where n is the average molar ratio of peptide per antibody

3. Peptide-to-Antibody Ratio and Characterization by HPLC

Peptide does not have any unique UV absorbance that allows easy calculation of the average peptide-to-antibody ratio based on the HPLC and UV analysis. The peptide-to-antibody ratio can be assayed by mass spectrometry or based on the amount of maleimide groups on the antibody before and after reaction using the maleimide assay kit (Product Number: CM90002 from CellMosaic, See Step C10).

To check the homogeneity and aggregation properties of the conjugate, you can analyze the conjugate by size exclusion chromatography (SEC) HPLC. If you do not have access to such a setup, you can send your sample to CellMosaic for analysis.

4. Recommended Storage Conditions

Based on our preliminary data, the antibody-peptide conjugate made with this kit is relatively stable at 2-8°C. For long-term storage, the conjugate can be stored at < -20°C. The stability of your conjugate may be different due to your antibody and should be checked by HPLC.

5. Submit Samples for HPLC Analysis

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

1) Dilute your un-conjugated antibody to 1 mg/mL in PBS buffer, then transfer 50 µL of the diluted solution to a 500 µL microcentrifuge tube. Label the vial properly.

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2) Dilute your conjugated antibody 5 times in PBS buffer, then transfer 50 µL of the diluted solution to a 500 µL microcentrifuge tube. Label the vial properly.
3) Ship your samples with a cold pack for overnight delivery.