CellMosaic™

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BSA-Peptide Conjugation Kit (CM52402x1 and CM52402x3) 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 labeling using this kit may be affected by many different variables, including but not limited to: purity and complexity of the peptide, differences in preparation techniques, operator abilities, 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 perform BSA labeling of one (CM52402x1) or three peptide samples (CM52402x3).

⚠ 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 (CM52402x1)	Quantity (CM52402x3)	Storage condition
Box 1	Activated BSA (red label)	CM52103	1x2 mg	3x2 mg	-20°C
Box 2	Solution A (green label)	CM01003	1.5 mL	3 mL	2–8°C
	Solution B (purple label)	CM01007	0.5 mL	0.5 mL	
	1xPBS buffer	CM02013	6 mL	20 mL	
	Centrifuge filter device	CM03CD030A	2	6	
	Collection tubes	N/A	4	6	
	1.5 mL centrifuge tube	N/A	1	3	
	0.5 mL centrifuge tube	N/A	1	3	
User Material	Cys-peptide	N/A	NOT PROVIDED (User Supplied Material. **0.15–0.6 µmol** for each reaction)		

Cys-peptide amount: This kit uses BSA modified with a high number of maleimide groups (20–40 average maleimides per BSA, please check the COA of each individual batch for the exact loading. **Note:** you cannot achieve loading greater than the number of maleimides). Peptide loading will be determined primarily by the amount of peptide added during the labeling. 0.15–0.6 µmole peptide will correspond to 5–20 equivalents of peptide over BSA.

Peptide to BSA ratio optimization: Peptide loading will be determined primarily by the amount of peptide added during the labeling if the equivalents of peptide over BSA is less than the maleimide groups per BSA. By balancing the amount of activated BSA and peptide added, the loading can be optimized to your target value (See Note in **Step A4**). You can experimentally measure the peptide loading by checking the % of peptide consumed during the labeling reaction (See HPLC or UV sampling and calculation in **Step A3** and **B6**).

Safety Information

Warning: some of the chemicals used can be potentially hazardous and can cause injury or illness. Please read and understand the Material Safety Data Sheets (MSDS) available at CellMosaic.com before you store, handle, or use any of the materials.

Labeling Chemistry

Bovine serum albumin (also known as BSA or "Fraction V") is a serum albumin protein derived from cows. BSA is often used as a carrier protein for immunization. CellMosaic has designed this personalized

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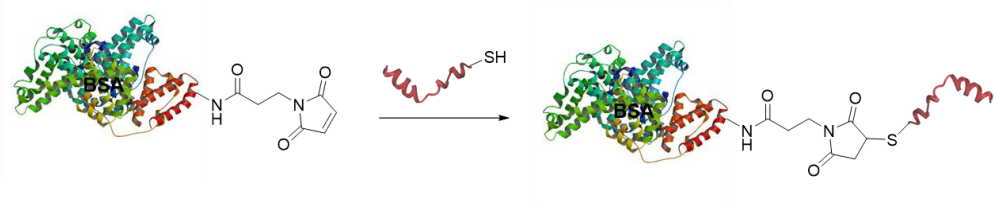
BSA conjugation kit to work with any peptide containing a reactive Cys group. The user supplies their own peptide. Using the kit components, the customer prepares the BSA-peptide conjugate by reacting the peptide (customer supplied) with activated BSA. One-step purification typically provides the resulting BSA-peptide at greater than 95% purity.

This kit provides materials to perform BSA labeling of one to three peptides. The total amount of activated BSA included in a reaction: 2 mg.

Key features of this BSA-peptide conjugation kit:

- High quality maleimide-activated BSA for the conjugation: >99% purity
- High maleimide groups per BSA for peptide loading: 20–40 (please check the COA of each individual batch for the exact loading)
- A single purification affords over 95% of the BSA-peptide conjugates
- Fast preparation: less than 1 h hands-on time
- All reagents and consumables are included, from preparation to purification
- Options to choose tailored custom services at CellMosaic prior to and after conjugation in your lab:
    - Prior to conjugation, you can supply your peptide information when you place your order and CellMosaic will give recommendations for the conjugation if your peptide has special features (complementary service);
    - After conjugation you can choose to send your sample to CellMosaic for HPLC analysis of the conjugate or peptide samples for determination of the peptide loading.



Requirement for Cys-peptide:

1. Amount: 0.15–0.6 µmol (5–20 equivalents of peptide over BSA)
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

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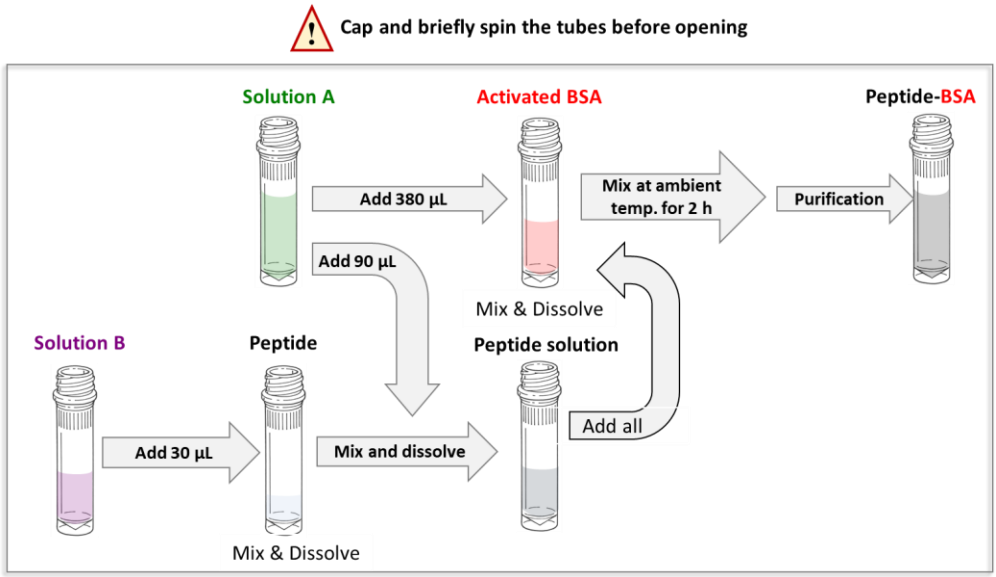
Protocol



Scheme 1. Schematic diagram of the workflow for preparing peptide-BSA conjugates.

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated)
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C or RT
- Balance

2. BSA Conjugation with Peptide

A1. Add 380 µL of **Solution A** (green label) to a tube containing **Activated BSA** (red label). Vortex for 30 seconds to 1 minute to dissolve the BSA.

> The following steps (**A2, A3, and A4**) are to be performed without any break. Cys peptide, once dissolved in solution, tend to oxidize quickly, and should be used immediately. Do not store any Cys peptide solution for later usage.

A2. Weigh 0.15-0.6 µmol of **Cys-peptide** into a 0.5 mL microcentrifuge tube. **Note:** the peptide in general is static 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 0.15 µmol and 0.6 µmol is acceptable (no need to adjust the volume of the dissolving solution).

Calculation for 0.15 µmol (target 5 peptides per BSA if all the peptides are labeled):

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Amount of Cys-peptide (mg) = Molecular weight of Cys-peptide x 0.00015

Calculation for 0.6 µmol (target 20 peptides per BSA if all the peptides are labeled):

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

A3. Add 30 µL of **Solution B** (purple label) to the centrifuge tube containing Cys-peptide from **Step A2.** Vortex for 30 seconds and centrifuge the tube to get all of the liquid down to the bottom. Open the cap and add 90 µL of **Solution A** (green label) to the tube. Vortex for 30 seconds or sonicate for a few minutes to ensure all of the solid is dissolved. Discard any unused **Solution B** as hazardous chemical waste **until the experiments are done**.

Tip for solubility check (Step A3): 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 some solid remains after a few minutes, 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.

HPLC or UV Sample (A3) for peptide loading measurement (Optional): If you are planning to experimentally determine the peptide loading, please follow the steps below:

- Transfer 6 µL of the peptide solution from **Step A3** (5% of the total amount) to a new centrifuge tube and label it as **sample A3.**
- For UV measurement, dilute the sample 10-50 times in 50% MeOH/water. Record the dilution factor DF(A3) and UV absorbance at 280 nm (A(A3)). Remember to use 50% MeOH/water as a blank for subtraction.
- For HPLC analysis, dilute the sample 5-20 times in 50% MeOH/water (detect at 205 and 280 nm). Record the dilution factor DF(A3) and HPLC Area (A(A3)).

A4. Transfer the solution from **Step A3** to the tube containing **Activated BSA** from **Step A1**. Pipette the solution up and down in the tube three times to mix. Incubate at room temperature for 2 hours (**Note:** if you use the decreased amount of **Activated BSA**, please pipette out the desired volume of **Activated BSA** from **Step A1** to the peptide solution from **Step A3** instead).

Peptide Loading Optimization: The desired peptide loading is specific to the project. Recommended target peptide loading is within 5–20 peptides per BSA. Your target loading can be adjusted by either changing the moles of peptide added in Step A2 or the volume of activated BSA from **Step A1**. You can also change both.

Calculation of the theoretical loading if 100% peptide are labeled:

$$Peptide\ per\ BSA\ (Loading) = \frac{Peptide\ (umole) * 12667}{Activated\ BSA\ (volume)}$$

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3. Purification to Remove Excess Peptide

B1. Place the centrifuge tube from **Step A4** containing the reaction mixture into the centrifuge rotor and counterbalance with a similar device. Spin **the centrifuge tube** at 10,000 x g for 2 minutes.

B2. Insert the **Filter Device** into one of the provided collection tubes (micro-centrifuge tube with the cap attached). Transfer and divide the supernatant from **Step B1** into two **Centrifuge Filter Devices**. Add 250 µL of **PBS Buffer** to make up the total volume to 500 µL in each filter device and cap it.

B3. Place the assembled **Filter Devices** into the centrifuge rotor, aligning the cap straps toward the center of the rotor. Spin the assembled **Filter Devices** at 14,000 x g for 8 minutes to concentrate down to < 100 µL (Spin time depends on many factors. A typical spin time for a 500 µL sample is approximately 8 to 20 minutes. The typical volume is ~40 µL of residual fluid in each filter device after spinning for 8 minutes on an Eppendorf 5417R at 4°C).

B4. Remove the assembled devices from the centrifuge and separate the **Filter Device** from the collection tube. Transfer the filtrate from the collection tubes 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 µL of **PBS Buffer** to make up the total volume to 500 µL in each filter device and cap it. Place the two assembled **Filter Devices** into the centrifuge rotor, aligning the cap strap toward the center of the rotor. Spin the assembled **Filter Devices** at 14,000 x g for 8 minutes.

B6. If the MW of your peptide is <3000 Da, repeat **Step B5** two times.

If the MW of your peptide is between 3000-5000 Da, repeat **Step B5** three times.

If the MW of your peptide is >5000 Da, repeat **Step B5** four times.

HPLC or UV Sample (B6) for peptide loading measurement (Optional): If you are planning to experimentally determine the peptide loading, please follow these steps:

- Combine the first three purification filtrates from **Steps B4–B6** and label it as **sample B6.** Record the total volume of the filtrate (~2.4 mL).
- For UV measurement, record the absorbance of the sample at 280 nm without dilution (A(B6)). Remember to use PBS buffer as a blank for subtraction.
- For HPLC analysis, inject the sample without dilution and detect the peak at 205 and 280 nm. Record the HPLC area (A(B6)).

Calculation of the peptide to BSA ratio:

$$Peptide\ per\ Ova = \frac{P}{B} \times \left(1 - \frac{A(B6) \times V(B6)}{A(A3) \times DF(A3) \times 6}\right) \times 7996$$

A(A3): UV absorbance at 280 nm or HPLC area of sample A3 (blank subtracted)
A(B6): UV absorbance at 280 nm or HPLC area of sample B6 (blank subtracted)
DF(A3): dilution factor of sample A3; **V**: total volume of filtrate B6

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P: total amount of peptide added in **Step A2** (µmole)
B: total volume of activated BSA added in **Step A4** (µL)

B7. To recover the conjugates, place the **Filter Device** upside down in a clean **Collection Tube**. Place in the centrifuge, aligning the open cap towards the center of the rotor. Spin for 2 minutes at 1,000 x g to transfer the conjugates from the **Filter Devices** to the **Collection Tubes.**

B8. Transfer the conjugates from the two **Collection Tubes** to a 1.5 mL micro-centrifuge tube.

B9. Rinse each **Collection Tube** with 50 µL of **PBS Buffer** and transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step B8**.

B10. Add 100 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 B8**. Add **PBS Buffer** to make the total volume of the sample 400 µL and cap it (use the pipetman to measure the total volume).

B11. Vortex the combined protein sample for 30 seconds and then centrifuge to ensure no liquid is in the cap.

BSA-Peptide is Ready for Your Experiment

Tip: The approximate concentration of **the BSA-peptide conjugate** is 60 µM in 400 µL of PBS buffer (assuming 80% recovery). You can also determine the concentration using a UV/Vis spectrophotometer. For downstream applications, avoid using any buffer that contains free thiol (e.g., Cys).

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Other Considerations

1. Concentration Determination

To determine the concentration, dilute your BSA-peptide from **Step B11** with 1x PBS buffer. Measure the UV absorbance of the BSA-peptide at 280 nm (A_{280}) using a UV spectrometer and calculate the concentration using the following formula:

Concentration (µM) of the dilute sample = (A_{280})/(L x ε)

Where A_{280} is the absorbance of BSA-peptide conjugates at 280 nm; L is the UV cell path length (cm); and ε is the extinction coefficient of the BSA-peptide conjugate at 280 nm.

If you know the sequence of the peptide and the average molar ratio of peptide per BSA, you can calculate ε as described in the following equation (Gill, S.C. and von Hippel, P. H. *Anal. Biochem.* **1989***, 182***,** 319–326; Pace, C.N., *et al. Protein Sci.* **1995***, 4,* 2411–2423):

ε = m x ((nW x 5500)+(nY x 1490)+(nC x 125))+43824

Where m is the average molar ratio of peptide per BSA. If you do not know the molar ratio, you can use the amount of peptide added.

Where n is the number of corresponding residues present in the peptide: tryptophan (W), tyrosine (Y), and cysteine (C).

If you are using a 1 cm UV cell, you can dilute the BSA-peptide 10-20 times to get a good reading.

2. MW Calculation

Calculation of the MW of the conjugate:

MW(conjugate) = m x MW(peptide) + 66400

Where m is the average molar ratio of peptide per BSA.

3. Recommended Storage Conditions

For long-term storage, BSA-peptide conjugates can be stored frozen at -20°C.

4. Peptide-to-BSA Ratio

Peptide loading can be measured experimentally by checking the % of peptide consumed during the labeling reaction. Please see HPLC or UV sampling and calculation of peptide-to-Ova ratio in **Step A3** and **B6**.

5. Sample Submission for HPLC Analysis

If you are submitting samples to CellMosaic for C18 HPLC analysis for sample loading and SEC HPLC for conjugate analysis, please follow these instructions:

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1) Go online: https://www.cellmosaic.com/hplc-analysis/, select C18 HPLC Analysis (Product# AS0021) and SEC HPLC Analysis (Product# AS0023), choose the quantity (number of samples - bulk discounts are available for multiple samples), and submit the order. Alternatively, you can email info@cellmosaic.com for a quote and to place the order.
2) For peptide loading determination by C18 HPLC, transfer sample **A3** and **B6** to a micro-centrifuge tube with screw cap and label the vial properly.
3) For conjugate purity analysis by SEC HPLC, mix 10 µL of your conjugate from **Step B11** with 40 µL of 1x PBS buffer into a micro-centrifuge tube with screw cap. Label the vial properly.
4) Ship your samples with a cold pack for overnight or 2nd day delivery.