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PerKit™ BSA Universal SM Acid Conjugation Kit (CM52425) 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

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 BSA conjugation kit to work with any small molecule containing a carboxylic acid (-COOH) functional group. The kit provides materials to conjugate 5 mg of BSA.

⚠ 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	Storage condition
Box 1	Reagent A (yellow label)	CM10004.2	1 unit	-20°C
	Reagent B (green label)	CM10003.1	1 unit	
Box 2	BSA (dark red label)	CM14001	5 mg (protein content)	2-8°C
	Solution A (blue label)	CM01008	0.5 mL	
	Buffer A (orange label)	CM02001	1 mL	
	Buffer B (sky blue label)	CM02006	1 mL	
	PBS buffer (grey label)	CM02013	30 mL	
	Desalting Column	CM03SG25	1	
	1.5 mL Centrifuge Tube	CM03CT2	2	
	5 mL Centrifuge Tube	CM03CT11	1	
User Material	Small Molecule Acid	N/A	NOT PROVIDED (User Supplied Material, **≥3.6 µmol**)	

Safety Information

Warning: Some of the chemicals used can be potentially hazardous and 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 work with small molecules containing one carboxylic acid functional group. The user supplies the small molecule. Using the kit components, the user converts the carboxylic acid to an activated sulfo *N*-hydroxysuccinimide ester (NHS ester), followed by reaction with the surface amino groups of BSA to form a stable amide bond. The product is then purified to remove any unreacted small molecule acid.

Key features of this conjugation kit:

- Offers a simple and easy way to label BSA with any small molecule containing a carboxylic acid group
- Stable linkage
- Fast and easy preparation: 4 h preparation and <1 h hands-on time
- All reagents and supplies included for preparation and purification

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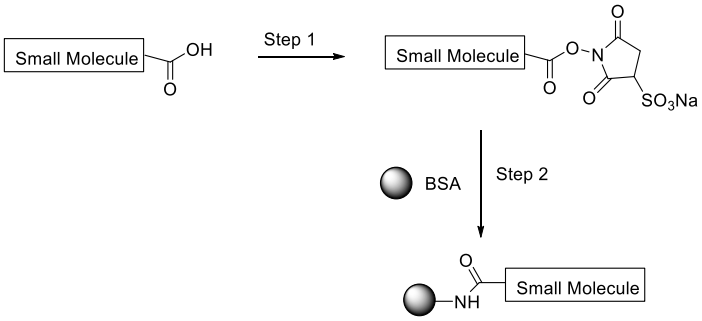
- More than 99% conjugated product (free of any unreacted small molecules)



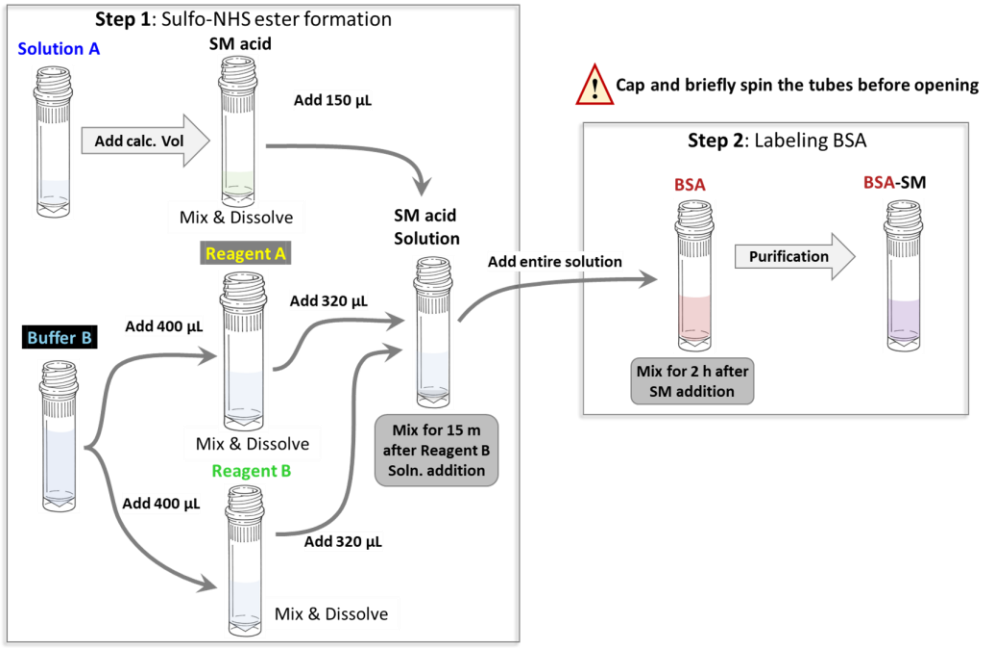
Requirement for small molecule:
1. Preferably > 90% pure
2. Total amount: ≥ 1.8 µmol
3. Preferably contains only one aliphatic carboxylic acid (-COOH)
4. Absence of primary or secondary amine groups

Note: the presence of a hydroxide (-OH) group will not affect the labeling.

Support

Customer can request a recommendation for the conjugation if the small molecule has a special feature. CellMosaic also provides additional support services to customers who need help analyzing the final conjugates by HPLC.

Protocol



Scheme 1. Schematic diagram of the workflow for preparing BSA small molecule conjugates.

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1. Lab Instrumentation Needed

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

2. Prepare Site and Reagents for Labeling Experiment

A1. Remove Box 1 containing **Reagent A** (yellow label) and **Reagent B** (green label) from the freezer and warm to RT.
A2. Remove Box 2 from the refrigerator.
A3. Set the temperature of the incubator or shaker to 25°C.

3. Sulfo-NHS Ester Formation (Step 1)

Items needed: Small Molecule Acid (user supplied), Reagent A (CM10004.2, yellow label), Reagent B (CM1003.1, green label), Solution A (CM01008, blue label), Buffer B (CM02006, sky blue label)

B1. Weigh at least 3.6 µmol (but no more than 20 µmol) of the small molecule acid into a clean 1.5 mL micro-centrifuge tube. Try to weigh at least 1 to 2 mg to obtain an accurate reading. Record the weight.

Calculation 1 for SM Acid Amount:

$$Amount\ of\ the\ minimum\ SM\ (mg)\ needed = MW\ \times 0.0036$$

B2. Spin the centrifuge tubes containing Reagent A (yellow label), Reagent B (green label), Solution A (blue label), and Buffer B (sky blue label) before opening them.

B3. Transfer calculated amount of Solution A to the centrifuge tube containing small molecule acid from **Step B1**. Vortex for 30 seconds to ensure all of the solid is dissolved and then spin down.

Calculation 2 for Solution A Volume:

$$Vol.\ of\ Solution\ A\ (\mu L) = \frac{Amt\ SM\ (in\ mg)}{MW} \times 50000$$

B4. Transfer 150 µL of SM acid solution from **Step B3** to a clean 1.5 mL centrifuge tube.

B5. Transfer 400 µL of Buffer B (sky blue label) to the tube containing Reagent A (yellow label). Vortex for 30 seconds to ensure all of the solid is dissolved and then spin down.

B6. Transfer 320 µL Reagent A solution from **Step B5** to the tube containing small molecule acid from **Step B4.** Vortex for 30 seconds to mix and then spin down.

B7. Transfer 400 µL of Buffer B (sky blue label) to the tube containing Reagent B (green label). Vortex for 30 seconds to ensure all of the solid is dissolved and then spin down.

Tip for solubility check (Steps B3, B5, & B7): Check the bottom of the micro-centrifuge tube to ensure the solution is clear and free of any solid residue.

B8. Transfer 320 µL of Reagent B solution from **Step B7** to the tube containing small molecule acid and Reagent A from **Step B6.** Vortex for 30 seconds to mix and then spin down.

B9. Let the tube remain at room temperature for exactly 15 minutes (no need to mix).

Start Time: _____ End Time:_____

4. Labeling BSA (Step 2)

Items needed: Small Molecule Acid Solution from **Step B9**, BSA (CM14001, dark red label), Buffer A (CM02001, orange label)

C1. Briefly spin the tube containing BSA (dark red label). Add 460 µL of Buffer A (orange label**)** to the tube. Vortex for 30 seconds to 1 minute to dissolve the reagent, and then centrifuge to ensure no liquid is in the cap.

C2. Transfer the entire solution from **Step B9** to the BSA solution. When you add the small molecule acid solution, place the pipette tip inside the BSA solution and then dispense the small molecule solution slowly while swirling the pipette tip.

Degree of SM Acid labeling (DOL): If you add the entire volume of the activated SM acid solution, you will obtain an average 3-10 SM acid per BSA. If the SM acid is very hydrophobic, you can decrease the volume of SM acid solution that is added to avoid precipitation of the conjugate.

C3. Cap the centrifuge tube. Mix at 25°C or room temperature 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 the centrifuge is capped properly. If you do not have any of this equipment, you can let the centrifuge tube sit on the bench with manual mixing by pipetting every 20 minutes.

Time-saving tip: While waiting for the reaction to complete, you can move on to **Step D1** and equilibrate the column for purification.

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5. Purification of Conjugate

Items needed: Desalting Column (CM03SG25), PBS Buffer (CM02013, grey label), 5.0 mL Centrifuge Tube (CM03CT11), SM-labeled BSA Solution from **Step C3**.

D1. Securely attach the Desalting Column to a support stand, lab frame, or any support rod. Remove the top and bottom caps from the column and allow the excess liquid to flow through by gravity. Collect the liquid in a flask.

D2. Add 5 mL of PBS Buffer and allow the buffer to completely enter the gel bed by gravity flow.

D3. Repeat **Step D2** five times.

D4. Spin the SM-labeled BSA solution from **Step C3** to ensure there is no liquid in the cap before opening it. Add the entire BSA solution to the column. Allow the sample to enter the gel bed completely.

D5. Add 1250 µL of PBS Buffer and allow the liquid to enter the gel bed completely.

D6. Place a 5.0 mL centrifuge tube under the column. Add 2.25 mL of PBS Buffer to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

D7. Label the tube as your product.

D8. Determine the concentration by UV/Vis spectrophotometry (see Other Considerations).

D9. Store the conjugate at 2−8°C for immediate usage. Aliquot and store the conjugate in a freezer at < -20°C for long-term storage.

Conjugate is Ready for Your Experiment

- **Specification for your product:** SM-labeled BSA with an average DOL of 3-10. The actual DOL will depend on the activities of the carboxylic acid of your SM. A typical batch contains over 99% conjugated product by SEC and is free of any unreacted SM. The approximate concentration of the BSA is 1.78 mg/mL in PBS buffer assuming 80% recovery.

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Other Considerations

1. Concentration Determination for Small Molecule Labeled BSA

To determine the concentration of the conjugate, dilute your conjugate from **Step D7** with PBS buffer. Measure the UV absorbance of the conjugate at 280 nm using a UV spectrometer and calculate the concentration based on the following formula:

$$Concentration\ (mg/mL)\ of\ the\ dilute\ sample = \frac{(A280)}{L(0.66 + n \times \varepsilon(280nm))}$$

Where **L** is the UV cell path length (cm); **n** is the average loading of small molecule; and **ε** is the extinction coefficient of your small molecule ($cm^{-1}mg^{-1}mL$) at 280 nm as appropriate. If the small molecule has only weak or no UV absorbance, you can use 0.

2. MW Calculation

Calculation of the MW of the conjugate:

$$MW(Conjugate) = n \times (MWs - 18) + 66400$$

Where **n** is the average loading of the small molecule (use 6 as average) and MWs is the MW of the small molecule.

3. Degree of Labeling (DOL) Calculation and Characterization by UV and MS

DOL can be measured by mass spectrum analysis. If the small molecule has a characteristic UV absorbance that does not overlap with the UV absorbance of BSA or has a considerably higher extinction coefficient than BSA at the same wavelength, you can use it for the calculation of the DOL. Otherwise, you can assume 3-8 small molecules per BSA for your conjugate if you add the standard amount of small molecule in the protocol during the labeling reaction.

4. Characterization of Conjugate by Reversed Phase HPLC

If your small molecule is very hydrophobic, standard reversed phase HPLC such as C18, C8, or C4 (preferred column) can be used to check whether a BSA is labeled with small molecule. If you do not have access to an HPLC facility, you can send your sample to CellMosaic for analysis.

5. Characterization of Conjugate by SEC HPLC

If you are concerned with aggregation, you can use size exclusion chromatography (SEC) to check the extent of aggregation. SEC separates the conjugates by apparent MW or size in aqueous solution. The larger the MW of the conjugate, the earlier it elutes. By comparing the SEC profile of the unlabeled protein and conjugate, you can estimate how much aggregation is in the conjugate. CellMosaic offers two SEC standards (Product #: CM92004 and CM92005) for our customers to use with any SEC column. The CM92004 product sheet contains all of the information and methodology you need to run an SEC HPLC analysis. If you do not have access to an HPLC facility, you can send your sample to CellMosaic for analysis.

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6. Recommended Storage Conditions

Recommended storage of the conjugate is at 2-8°C for short-term usage.

If you need to store the conjugate for a longer period, aliquot and store the conjugate in a freezer at < -20°C or lyophilize to dryness. Avoid repeated freeze and thaw cycles.

7. Submit Samples for HPLC Analysis

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

1) Go online: https://www.cellmosaic.com/hplc-analysis/, select SEC HPLC Analysis (Product# AS0023) and C4 HPLC Analysis (Product#: AS0027). Choose the quantity (i.e., 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) Transfer 50 µL of conjugate to a 500 µL microcentrifuge tube and label the vial properly.
3) Ship your samples with a cold pack for overnight delivery.