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PerKit™ BSA Small Molecule Acid Conjugation Kit (CM52403) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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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 small molecule containing a carboxylic acid functional group onto any protein.

Name	Part #	Quantity	Storage condition
BSA (dark red)	CM14001	5 mg (protein content)	2-8 °C
Reagent A solution (white)	CM10001	1 unit (40 µL)	2-8 °C, dry
Reagent B (purple)	CM10002	1 unit	2-8 °C, dry
Solution A (blue)	CM01006	0.5 mL	2-8 °C
Reaction Buffer (orange label)	CM02001	1 mL	2-8 °C
1 x PBS buffer (grey label)	CM02013	20 mL	2-8 °C
Desalting Column	CM03SG10	1	2-8 °C
2 mL Centrifuge Tube	N/A	1	2-8 °C
0.5 mL Centrifuge Tube	N/A	2	2-8 °C
Small Molecule Acid	N/A	NOT PROVIDED (User Supplied Material, 20 µmol)	

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 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 *N*-hydroxysuccinimide ester (NHS ester), followed by reaction with the surface amino groups of BSA to form a stable amide bond. The final product is desalted to remove any unreacted small molecule acid.

Key features of this conjugation kit:

- Offers a simple and easy way to label BSA with small molecules containing carboxylic acid
- Fast and easy preparation: 4 h preparation and less than 30 minutes hands-on time
- Target average degree of loading: 3-6
- All reagents and supplies included for preparation and purification
- Over 90% pure conjugated products by SEC

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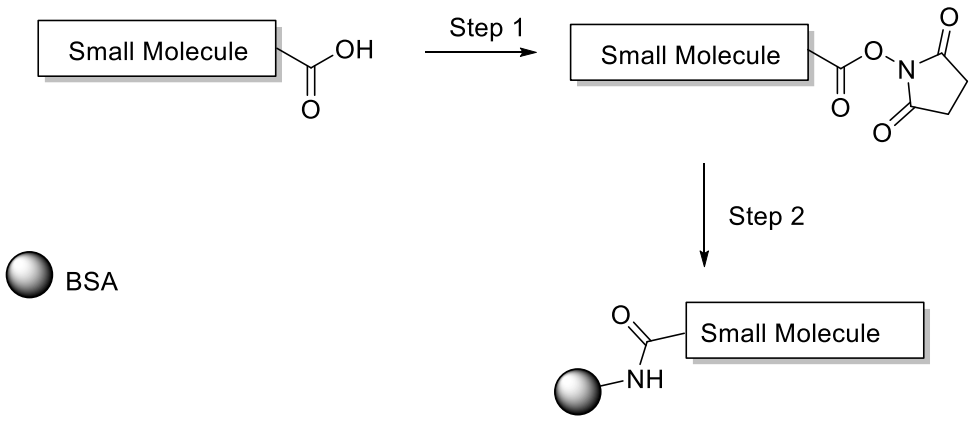
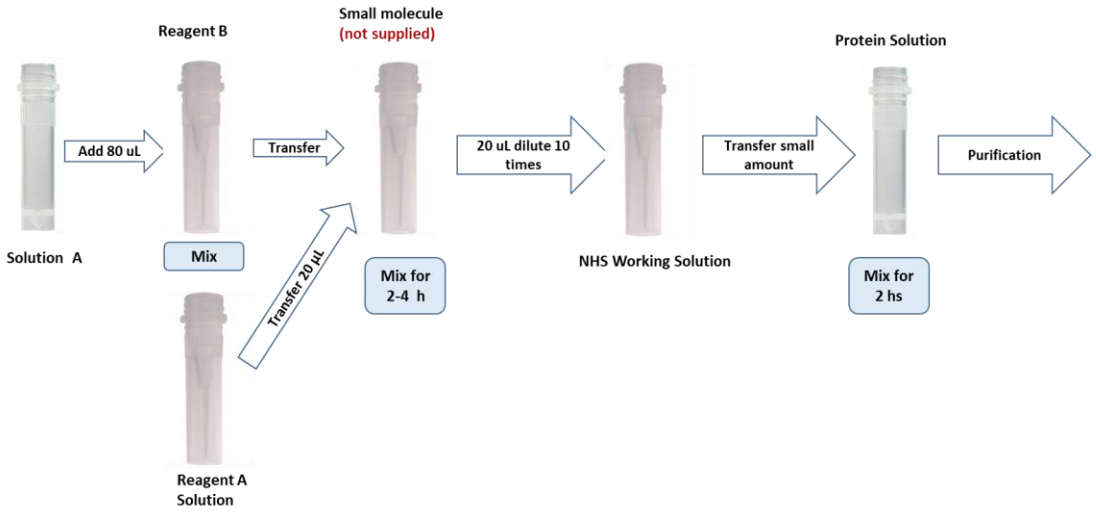


Requirement for small molecule:
1. Preferably > 90% pure
2. Total amount: 20 µmol
3. Absence of primary or secondary amine groups
4. Non-hindered aliphatic carboxylic acid
5. For molecule containing aromatic carboxylic acid, hindered aliphatic carboxylic acid, or hydroxyl groups, please consult CellMosaic prior to conducting the experiment.

Support

Customer can request a PerKit™ sheet containing the calculation, chemical structure, MW of the customer's final conjugate, and a recommendation for the conjugation if the molecule has a special feature or a low amount of small molecule is available. CellMosaic also provides additional support services to customers who need help analyzing the intermediates and final conjugates.

Protocol



Scheme 1. Schematic diagram of the work flow for preparing BSA-small molecule conjugates

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1. Lab Instrumentation Needed

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

2. NHS Ester Formation (20 µmol scale)

A1. Weigh 20 µmol of **Small Molecule** into a clean 0.5 mL micro-centrifuge tube.

Calculation: Amount of small molecule (mg) = Molecular Weight (MW) of small molecule x 0.02

A2. Open the plastic bag for NHS ester formation. Spin the centrifuge tubes containing **Reagent A solution** (white label), **Reagent B** (purple color insert), and **Solution A** (blue color insert) to ensure all the solid or solution is at the bottom of the tube before opening it.

A3. Transfer **20 µL** of **Reagent A solution** (white label) to the tube containing small molecule from **Step A1**. Vortex for 30 seconds or sonicate for a few minutes to ensure most of the solid is dissolved (**Note:** if there are some solids left, that is fine for this step). Centrifuge the tube to get all of the liquid down to the bottom.

A4. Add **80 µL** of **Solution A** (blue color insert) to **Reagent B** (purple color insert). Vortex for 30 seconds or sonicate for a few minutes to ensure all of the solid is dissolved. Transfer the entire solution to the tube containing small molecule from **Step A3**.

Tip for solubility check (Step A3, A4, & A5): It may take a while for your compound to fully dissolve. In general, most of the compound should be able to dissolve. Check the bottom of the micro-centrifuge tube to ensure the solution is clear and free of any solid residue.

Tip for precipitation check (Step A7): Place the tube at a 45-degree angle and see if the solution can flow freely. Remove the tape label if necessary.

A5. Vortex for 30 seconds or sonicate for a few minutes to ensure all of the solid is dissolved. Centrifuge the tube to get all of the liquid down to the bottom. (**Note:** some NHS ester formations are very fast, you might notice colorless solid precipitate out immediately after you mix up the solution).

A6. Incubate the mixture at RT for **2 h**.

A7. Remove the centrifuge tube from the incubator to check if there is any clear solid precipitated out. If there is solid precipitated out, move on to the next step. If not, leave the centrifuge tube in the incubator for another 2 h. (**Note**: If there is no solid precipitated out after 4 h of reaction, please consult with CellMosaic for an alternative method).

A8. Add **180 µL** of **Solution A** to a clean 0.5 mL micro-centrifuge tube. Discard any unused **Solution A** as hazardous chemical waste **until the experiments are done**.

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A9. Spin the centrifuge tube from **Step A7** to ensure there is no liquid in the cap before opening it. Pipette **20 µL** of solution using a very fine pipette tip (gel loading tip works great) and ensure there is no solid on the side of the tip. Transfer the liquid to the centrifuge tube containing the Solution A from **Step A8**.

A9. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap.

3. Conjugation with BSA

B1. Briefly spin the tube containing **BSA** (dark red label). Add 0.66 mL of **Reaction Buffer** (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.

B2. Add NHS solution from **Step A9** and **Reaction Buffer** to the 1.5 mL micro-centrifuge tube containing the solution of **BSA**. Set up the reaction as in the following table based on your target degree of labeling (DOL). Add the NHS ester solution first. When you add the NHS ester solution, place the pipette tip inside the sample solution and then dispense the NHS ester slowly with constant stirring by pipette tip. Make sure the NHS ester is mixed properly before adding the next drop. If your compound is very hydrophobic and you notice some solid precipitate out after you finish adding all the NHS ester solution, add **Solution A** instead of **Reaction Buffer** instead to dissolve the precipitate. If the solution is not clear after adding Solution A, move on to step **B3**. The precipitate will be removed during the purification.

	NHS ester solution from Step A7 (µL)	Reaction Buffer or Solution A (µL)
Target DOL: 1-3	22.5	67.5
Target DOL: 3-6	**45**	**45**
Target DOL: 4-8	67.5	22.5
Target DOL: 6-11	90	0

B3. Incubate the solution from **Step B2** at RT for 2 h.

4. Purification of Conjugate

C1. Securely attach the **Desalting Column** to support stands, lab frames, 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.

C2. Add 5 mL of **PBS buffer** and allow the buffer to completely enter the gel bed by gravity flow.

C3. Repeat **Step C2** twice.

C4: Add the sample from **step B3** to the column. Allow the sample to enter the gel bed completely.

C5. Add 250 µL of **PBS buffer** and allow the liquid to enter the gel bed completely.

C6: Place a 2 mL micro-centrifuge tube under the column. Add 1.25 mL of PBS buffer to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.

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C6: Label the tube as your product. Determine the concentration by UV/Vis spectroscopy. Store your conjugate at 4°C. For long term storage, you can freeze your product.

Conjugate is Ready for Your Experiment

Specification for your product: small molecule labeled BSA and free or less than 5% of unreacted small molecules. The approximate concentration of the BSA is 3.2 mg/mL in PBS buffer assuming 80% recovery. You can determine the concentration by UV/Vis spectroscopy (extinction coefficient at 280 nm for BSA is 43,824 $M^{-1}cm^{-1}$)