CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

PerKit™ Antibody Small Molecule Acid Conjugation Kit (CM51403) User Reference Guide

Contents

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

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.

For Research Use Only. Not for Use in Diagnostic Procedures.

The information in this document is subject to change without notice. CellMosaic assumes no responsibility for any errors that may appear in this document. In no event shall CellMosaic be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

NOTICE TO PURCHASER: LIMITED LICENSE

The purchase of this product includes a limited, non-transferable license to use this product to practice the labeling methods using the reagents solely for the purchaser's research activities. The license granted herein is personal to the original purchaser and may not be transferred to any other party outside the purchaser's company. No other right or license is conveyed or granted either expressly, by implication or by estoppel, to resell or repackage this product. Further information can be obtained by contacting:

Director of Licensing
c/o CellMosaic, Inc.
10-A Roessler Road, Woburn, MA 01801.
Phone： 781-463-0002
Fax： 781-998-4694
E-mail: info@cellmosaic.com

CellMosaic™

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Kit Components

This kit provides materials to conjugate a small molecule containing a carboxylic acid functional group onto any antibody.

Name	Part #	Quantity	Storage condition
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	8 mL	2-8 °C
1 x PBS buffer (grey label)	CM02013	20 mL	2-8 °C
Centrifugal Filter Device (10K)	CM03CD010A	2	2-8 °C
Desalting Column	CM03SG10	1	2-8 °C
Collection Tubes	N/A	2	2-8 °C
1.5 mL Centrifuge Tube	N/A	2	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)	
Antibody (IgG)	N/A	NOT PROVIDED (User Supplied Material, 3 mg)	

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

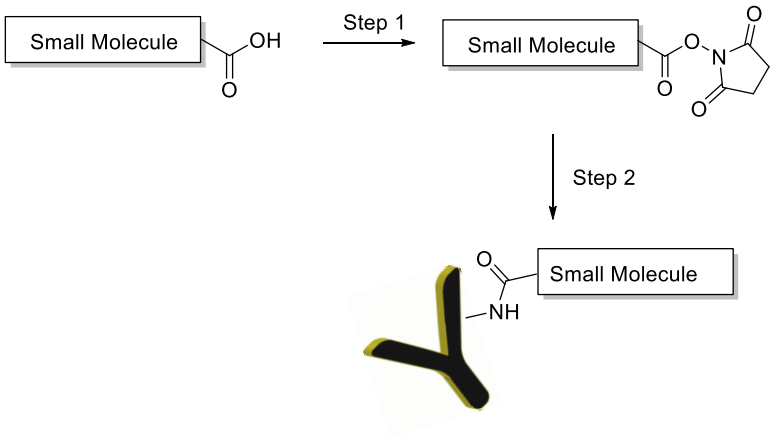
The kit is designed to work with small molecules containing one carboxylic acid functional group. The user supplies the small molecule. Using the kit components, the user converts the carboxylic acid to an activated *N*-hydroxysuccinimide ester (NHS ester), followed by reaction with the surface amino groups of the antibody 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 antibody 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

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com



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.

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

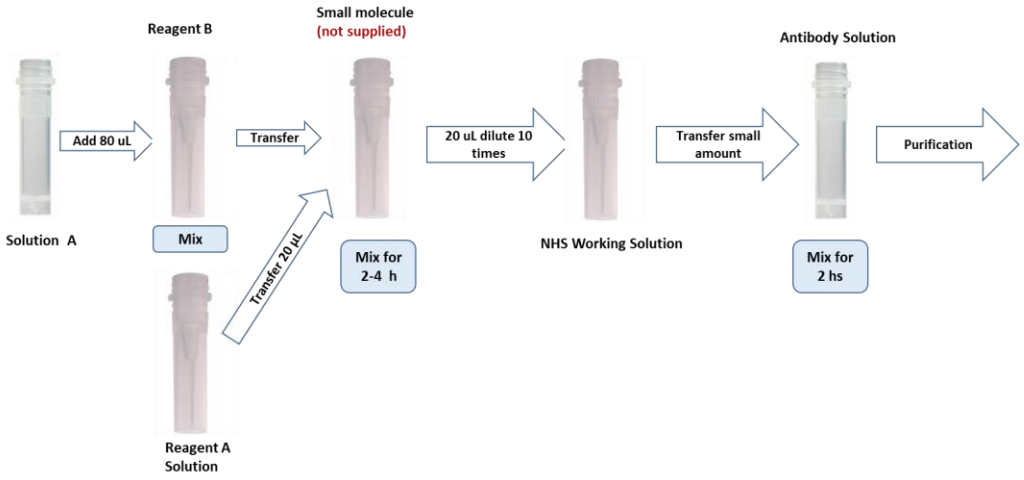
Support

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 antibody and small molecule is available. CellMosaic also provides additional support services to customers who need help analyzing the intermediates and final conjugates.

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

Protocol



Scheme 1. Schematic diagram of the work flow for preparing antibody-small molecule 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. Preparation of Antibody Samples for Conjugation

Total amount of antibody used for the conjugation is 3 mg.

A1. Perform the step based on the following conditions.

- ✓ 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 **Reaction Buffer** 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 **Reaction Buffer** to make up the total volume to 500 µL in each device and cap it.
- ✓ 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 Step **A1-A4** until all of the antibody sample goes into the **Filter Device**. Move on to Step **A5**. Add **Reaction Buffer** to make up the total volume to 500 µL per each device for the last refill.

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

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 10 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 10 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 to the collection tube. Add 400-450 µL of **Reaction Buffer** to make up the total volume to 500 µL. Then place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device. Spin the device at 14,000 x g to concentrate to **< 100 µL**. 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.**

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

A7. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube (use the pipetman to measure the approximate volume of the concentrated sample).

A8. Add 50 µL of **Reducing Buffer** 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 **Reaction Buffer** to the 1.5 mL micro-centrifuge tube from **Step A9** to make up the total volume of the sample to **340 ± 5 µL** and cap it.

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

Antibody Sample is Ready for Conjugation

3. NHS Ester Formation (20 µmol scale)

B1. 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

B2. 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.

B3. Transfer **20 µL** of **Reagent A solution** (white label) to the tube containing small molecule from **Step B1**. 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.

B4. 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 B3**.

B5. 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).

B6. Incubate the mixture at RT for **2 h**.

B7. 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).

Tip for solubility check (Step B3, B4, & B5): 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 B7): Place the tube at a 45-degree angle and see if the solution can flow freely. Remove the tape label if necessary.

B8. 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**.

B9. Spin the centrifuge tube from **Step B7** 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 B8**.

B9. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap.

NHS Ester is Ready for Conjugation

4. Conjugation with Antibody

C1. . Add NHS solution from **Step B9** and **Reaction Buffer** to the 1.5 mL micro-centrifuge tube containing the solution of **Protein** from **Step A11**. 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, you might also notice some solid precipitate out after you finish adding all the NHS ester solution. Add **Solution A** instead of Reaction Buffer afterwards to bring the precipitate back to solution. If the solution is not clear, move on to step **C2**. The precipitate will be removed during the purification.

CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801
USA

Phone: 781-463-0002
Fax: 781-998-4694
Email: info@cellmosaic.com
Website: www.cellmosaic.com

	NHS ester solution from Step B7 (µL)	Reaction Buffer or Solution A (µL)
Target DOL: 1-2	3	57
Target DOL: 2-3	6	54
Target DOL: 3-6	**12**	**48**
Target DOL: 4-8	24	36

DOL: The target DOL will depend on the number of Lys groups in your protein and the hydrophobicity of your small molecule. If your protein has limited Lys groups, the average DOL may be much lower. If your small molecule is very hydrophobic, you might also get lower DOL. If you are concerned with the DOL and your small molecule has some unique UV chromophore, you can use the UV-Vis spectrum to check the loading capacity. **Recommended to use 12 µL of NHS ester solution and target DOL 3-6.**

C2. Incubate the solution from **Step C1** at RT for 2 h (overnight reaction is OK for extended labeling).

Purification

5. Purification of Conjugate

D1. In a chemical hood, securely attach the **Desalting Column** to a support stand, a 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 E2** twice.
D4. Spin the labeled antibody solution from **Step C2** to ensure there is no liquid in the cap before opening it. Add the entire antibody solution to the column.
D5. Add 600 µL of **PBS buffer** and allow the liquid to enter the gel bed completely.
D6. Place a 1.5 mL centrifuge tube under the column. Add 0.9 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. Store your conjugate at 4°C.

Conjugate is Ready for Your Experiment

Specification for your product

The approximate concentration of the antibody small molecule conjugate is 1.67 mg/mL in PBS buffer assuming 50% recovery. You can determine the concentration by UV/vis spectrophotometry and the loading by HPLC or MS.