

PerKit™ Small Molecule Acid by Cy5 (CM62403) User Reference Guide

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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 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 perform cyanine 5 dye labeling of a small molecule containing a carboxylic acid functional group.

Name	Part #	Quantity	Storage condition
Cyanine 5 amine solution	CM62001	1 mg	2-8°C under dark
Reagent A solution	CM10001	1 unit (20 µL)	2-8°C, dry
Reagent B	CM10002	1 unit	2-8°C, dry
Solution A	CM01006	0.3 mL	2-8°C, dry
Small molecule acid	N/A	NOT PROVIDED (User Supplied Material, 20 µmol for each reaction)	

Safety Information

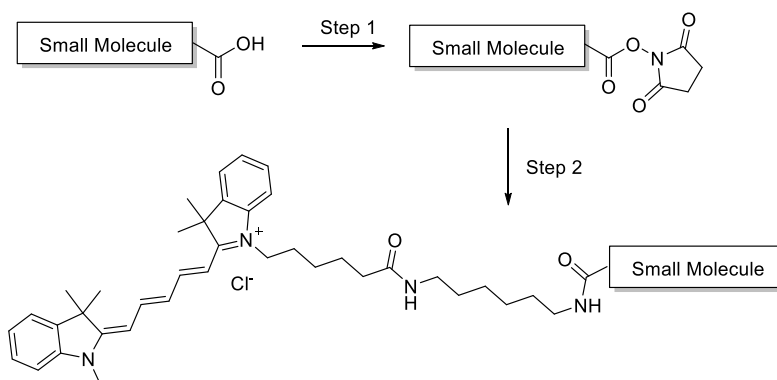
Warning: some of the chemicals used can be potentially hazardous and can cause injury or illness. Please read and understand the Material Safety Data Sheets (MSDS) available at CellMosaic.com before you store, handle, or use any of the materials.

Labeling Chemistry

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 an equivalent amount of the amine cyanine 5 dye.

Key features of this PerKit™:

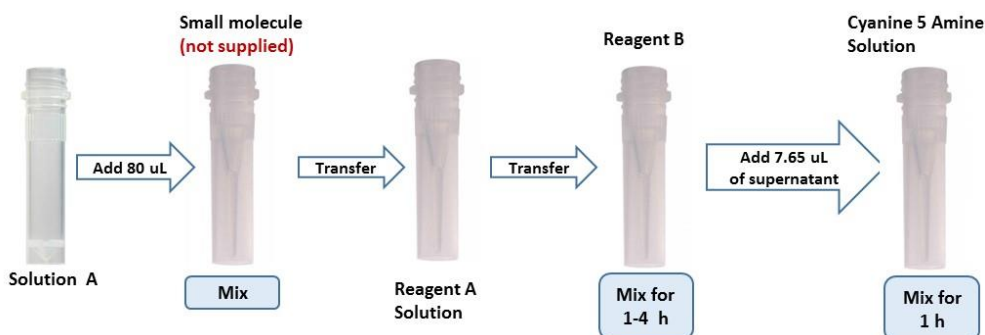
- Offers a simple and easy way to label small molecules with carboxylic acid
- Fast and easy preparation: 2hr preparation and less than 30 minutes hands-on time
- Quantitative reaction with stable bond
- All reagents included for preparation



Requirement for small molecule:

1. Preferably > 90% pure
2. Absence of primary or secondary amine groups
3. Non-hindered aliphatic carboxylic acid
4. For molecule containing aromatic carboxylic acid, hindered aliphatic carboxylic acid, or hydroxyl groups, please consult CellMosaic first prior experiment.

Protocol



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 0.5 mL micro-centrifuge tube and add 80 μL of **Solution A** (blue color insert). Vortex for 30 seconds or sonicate for a few minutes to make sure all of the solid is dissolved.

Calculation: Amt (mg) = Mw (small molecule) x 0.02

A2. Transfer the entire solution from **Step A1** to the tube containing **Reagent A solution** (white color insert). Vortex for 30 seconds and centrifuge the tube to get all of the liquid down to the bottom.

A3. Transfer the entire solution from **Step A2** to **Reagent B** (purple color insert). Vortex for 30 seconds and centrifuge the tube to get all of the liquid down to the bottom. Then incubate

Tip for solubility check (Step A1): 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 see if the solution is clear and free of any solid residue.

Tip for opening centrifuge tube after vortex: Always centrifuge the tube to make sure no liquid is in the cap.

Tip for precipitation check (Step A4): Place the tube at a 45 degree angle and see if the solution can flow freely.

the mixture at RT for 1 hr.

A4. 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 3 hr. (**Note:** If there is no solid precipitated out after 4 hours of reaction, please consult with CellMosaic for an alternative method).

NHS Ester is Ready for Conjugation

3. Conjugation with Cyanine 5 (1 mg scale)

B1. Spin the micro-centrifuge tube from **Step A4** to make sure there is no liquid in the cap before opening it. Pipette 7.65 µL of solution using a very fine pipette tip (gel loading tip works great) and make sure there is no solid on the side of the tip. Transfer the liquid to the centrifuge tube containing the solution of **cyanine amine** (orange color insert).

B2. Incubate the solution from **Step B1** at RT for 1 hr. The approximate concentration of the **conjugate** is 20 µM in biocompatible water miscible organic solvent.

Small Molecule-Cyanine 5 is Ready for Your Experiment

Other Considerations

1. Analysis

In general, the reaction will be completed within one hour. The progress of the reaction can be monitored by C18 reversed-phase HPLC using the following conditions:

Buffer A: 0.1% TFA in water

Buffer B: 0.1% TFA in Acetonitrile

Method: a linear gradient of AB solvent (5% B to 95% B in 12 minutes, then held at 95% B for another 3 minutes)

Flow rate: will be determined by your column (usually 1 mL/min).

Wavelength: 220 nm, 254 nm, 640 nm

Cyanine 5 amine: elute at 60% B if the dead volume be considered (see Appendix)

Conjugate: elute later than Cyanine 5 amine unless the small molecule is very hydrophilic.

2. Further Purification

Organic solvent can be removed by C18 cartridge desalting.

3. MW Calculation

Calculation of the MW of the conjugate:

$$\text{Mw}(\text{conjugate}) = \text{Mw}(\text{small molecule}) + 600.3$$

4. Spectra Properties of Cyanine 5

Excitation maximum: 646 nm

Extinction coefficient (ϵ): 250,000 L·mol⁻¹·cm⁻¹

Emission maximum: 662 nm

Fluorescence quantum yield: 0.2

5. Recommended Storage Conditions

For long-term storage, conjugates can be stored in the dark at -20°C.

Appendix: Typical Kit Performance Data (LC Analysis, CellMosaic)

Small molecule information: Fmoc-Gly-OH

Figure 1: C18 HPLC/UV analysis of cyanine 5 amine (detected at 640 nm)

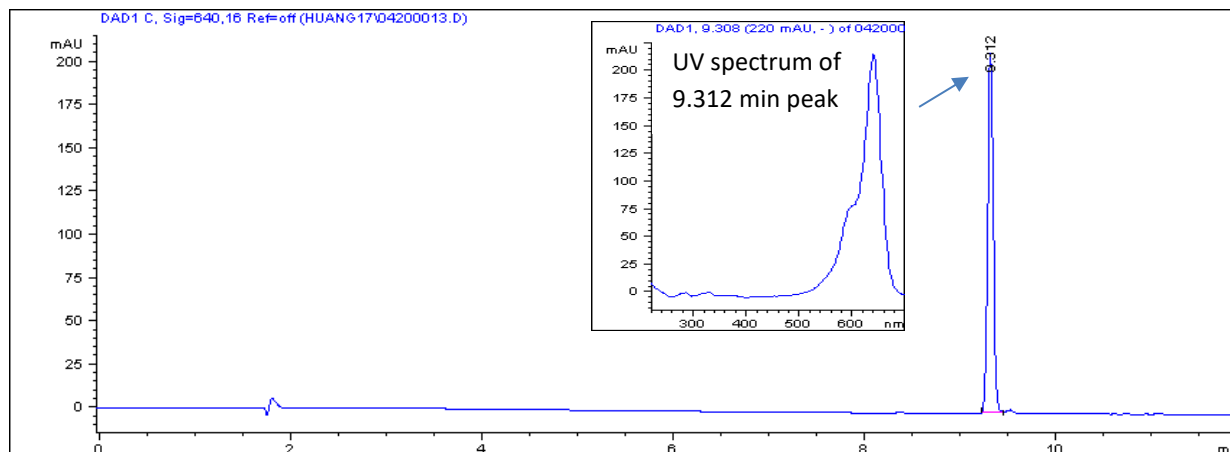
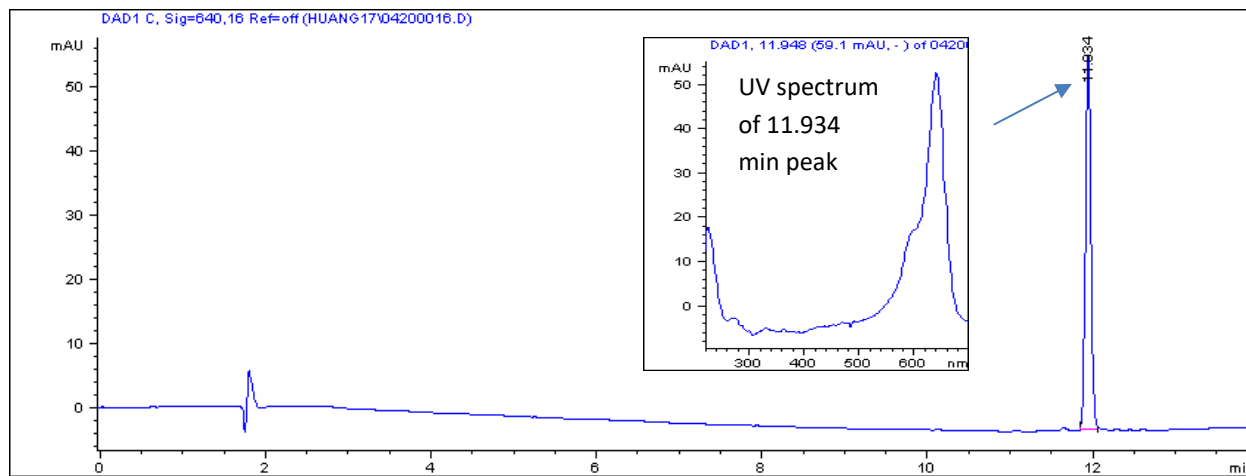


Figure 2: C18 HPLC analysis of the conjugation mixture of cyanine 5 amine with Fmoc-Gly-OH (1:1 ratio of cyanine 5 amine to Fmoc-Gly-OH was used). All cyanine reacted. A single product peak detected at 640 nm demonstrates the characteristic UV spectra of the Fmoc group and cyanine.



HPLC column: Agilent Eclipse XDB-C18 (5 μ m, 4.6x150 mm).

HPLC method: a linear gradient of AB solvent (5% B to 95% B in 12 minutes, then held at 95% B for another 2 minutes) at a flow rate of 1 mL/min

Column temperature: 25 °C.

Solvent A: 0.1% aqueous TFA

Solvent B: 0.1% TFA in acetonitrile.

UV detection: 640, 220, 254 nm.