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SepSphere™ Small Molecule Acid Immobilization Kit (CM71004) 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 immobilize a small molecule containing a carboxylic acid functional group onto amine-modified agarose beads.

Name	Part #	Quantity	Storage condition
Amine-modified 4% agarose beads in a 20 mL column	CM71603	5 mL	4 °C
Reagent A	CM10003	1 unit	-20°C, dry
Reagent B	CM10004	1 unit	-20°C, dry
Solution A	CM01008	1.5 mL	Ambient Temp
Reaction Buffer	CM02006	20 mL	Ambient Temp
Storage Buffer (0.02% NaN_3 in PBS)	CM02008	10 mL	Ambient Temp
15 mL centrifuge tube		1 tube	Ambient Temp
1.5 mL centrifuge tube		2 tubes	Ambient Temp
HPLC vial		2 vials	Ambient Temp
Small molecule acid	N/A	NOT PROVIDED (User Supplied Material, 50 µmol)	
Deionized water	N/A	NOT PROVIDED	

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 sulfo *N*-hydroxysuccinimide ester (NHS ester), followed by reaction with amine-modified agarose beads.

Key features of this SepSphere™ immobilization kit:

- Offers a simple and easy way to label small molecules with carboxylic acid
- Fast and easy preparation: 3 h preparation and less than 30 minutes hands-on time
- Quantitative reaction with stable bond
- All reagents and supplies included for preparation
- Use agarose beads with optimized amount of amine functional groups for loading to preserve the nature of the agarose beads

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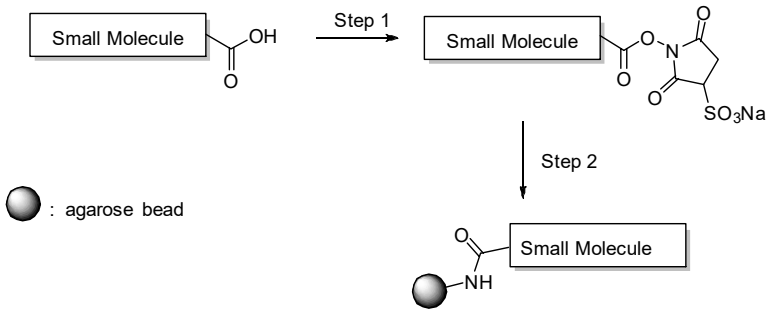
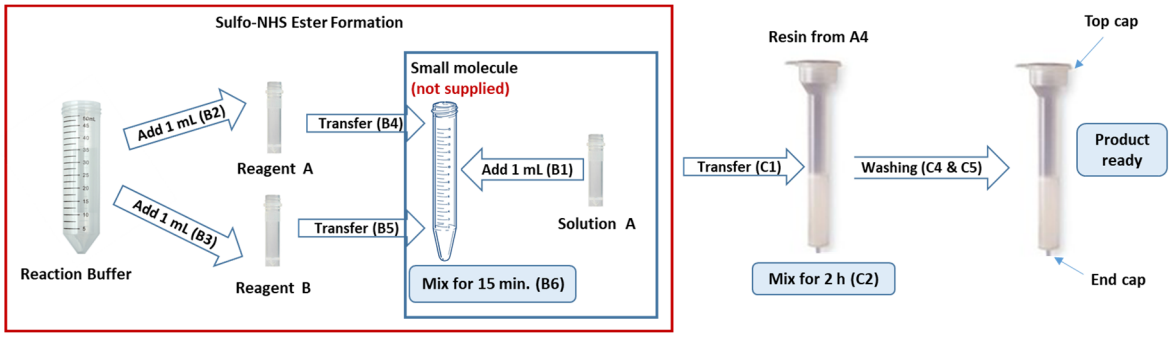


Requirement for small molecule:
1. Preferably > 90% pure
2. Absence of primary or secondary amine groups
3. Non-hindered aliphatic carboxylic acid
4. For molecule containing aromatic carboxylic acid, hindered aliphatic carboxylic acid, or hydroxyl groups, please consult CellMosaic prior to conducting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

Protocol



1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated)
- Pipettes and tips
- Timer
- Nutating mixer or 3-D Rocker
- Balance
- Vacuum filtration device

2. Filtration Setup

There are several ways to filtrate and wash the agarose beads

1) Vacuum filtration using Supelco Visiprep™ SPE vacuum manifold
2) A peristaltic pump, such as a variable flow mini-pump: attach the IN tubing to the bottom of the column and withdraw the liquid

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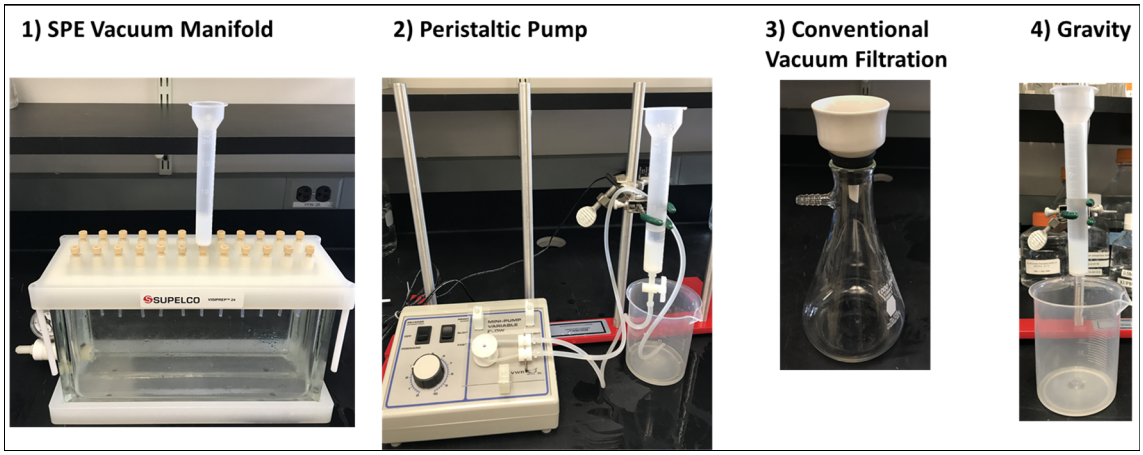
3) Traditional vacuum filtration with a Buchner funnel: transfer the beads to the funnel with a filter paper inside and draw the liquid by applying a vacuum to the side arm adaptor of the Buchner flask
4) Gravity or simple filtration (not recommended): filtration will be extremely slow.



3. Preparation of Agarose Beads

A1. Slightly centrifuge the column containing agarose beads to ensure no bead is in the top cap. Open the end cap and attach the column to a vacuum filtration device. Open the top cap and let the liquid drain.

A2. Wash the column with 10 mL of deionized water.

A3. Repeat **Step A2** two times.

A3. Wash the column with 5 mL of **Reaction Buffer** and completely drain the liquid.

A4. Securely cap the end of the column. Add 7 mL of **Reaction Buffer**.

Amine Agarose Bead is Ready for Immobilization

4. Sulfo-NHS Ester Formation (50 µmol scale)

B1. Weigh 50 µmol of **Small Molecule** into a 15 mL centrifuge tube and add 1 mL of **Solution A.** Vortex for 30 seconds or sonicate for a few minutes to ensure all of the solid is dissolved. Discard any unused **Solution A** as hazardous chemical waste.

Calculation: Amount of small molecule (mg) = Molecular Weight (MW) of small molecule x 0.05

B2. Add 1 mL of **Reaction Buffer** inside the tube containing **Reagent A** (white color insert). Vortex for 30 seconds and centrifuge the tube to get all of the liquid into the bottom.

B3. Add 1 mL of **Reaction Buffer** inside the tube containing **Reagent B** (purple color insert). Vortex for 30 seconds and centrifuge the tube to get all of the liquid into the bottom.

B4. Transfer the **Reagent A** solution from **Step B2** to the **Small Molecule solution in Step B1.**

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B5. Transfer the **Reagent B** solution from **Step B3** to the **Small Molecule solution in Step B1**. Vortex for 30 seconds and centrifuge the tube to get all of the liquid into the bottom.

B6. Incubate the mixture at room temperature for 15 minutes.

Sulfo-NHS Ester is Ready for Immobilization

5. Immobilization

C1. Add the entire NHS ester content from **Step B6** into the column containing agarose beads from **Step A4** and securely cap the column.

HPLC Sample C1 (Optional). If you are planning to remove the sample for HPLC analysis, please follow these steps: Mix the column for 30 sec, then centrifuge briefly to ensure no bead is in the cap. Open the top cap and transfer 200 µL of liquid into a 1.5 mL centrifuge tube. Centrifuge at 14,000 x g for 1 minute, then transfer 100 µL of the supernatant to an HPLC vial labeled **Sample C1**.

C2. Nutate the column at room temperature for 2 h.

C3. Slightly centrifuge the column to ensure no bead is in the cap. Open the end cap and attach the column to a vacuum filtration device. Open the top cap and let the liquid drain.

HPLC Sample C3 (Optional). If you are planning to remove the sample for HPLC analysis, please follow these steps: Centrifuge briefly to ensure no bead is in the cap. Open the top cap and transfer 200 µL of liquid into a 1.5 mL centrifuge tube. Centrifuge at 14,000 x g for 1 minute, then transfer 100 µL of the supernatant to an HPLC vial labeled **Sample C3**.

C4. Wash the column with 15 mL of deionized water.

C5. Repeat **Step C4** five times.

C6: Remove the column from the filtration device and cap the end of the column, add 10 mL of deionized water or PBS buffer. For long-term storage, add 10 mL of **Storage Buffer** instead.

Agarose Bead is Ready for Your Experiment

In general, the immobilization reaction is very fast and can be completed within 2 h. You do not have to analyze the reaction. The approximate loading of your small molecule on agarose is 10 µmol per mL of settled beads if all of the small molecules are loaded.

Specification for your product

Matrix: 4% beaded agarose (source: GE Life Sciences, Sepharose® 4B)
Particle size: 45 – 165 µm
Ligand concentration: ⩽ 10 µmole per mL of settled beads

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Other Considerations

1. Determine the Loading Capacity by HPLC

The amount of the small molecule loaded onto the resin can be calculated based on the amount of the small molecule consumed during the reaction. If you are familiar with HPLC, you can remove two samples and analyze them by HPLC. Alternatively, you can send the samples to CellMosaic for analysis on your behalf.

HPLC conditions

Buffer A: 0.1% TFA in water **Buffer B**: 0.1% TFA in acetonitrile
Method: 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).
Injection amount: 5 µL

Loading capacity

µmole per mL: ([HPLC area of Sample C1]-[HPLC area of Sample C3]) x 10 /[HPLC area of sample C1]

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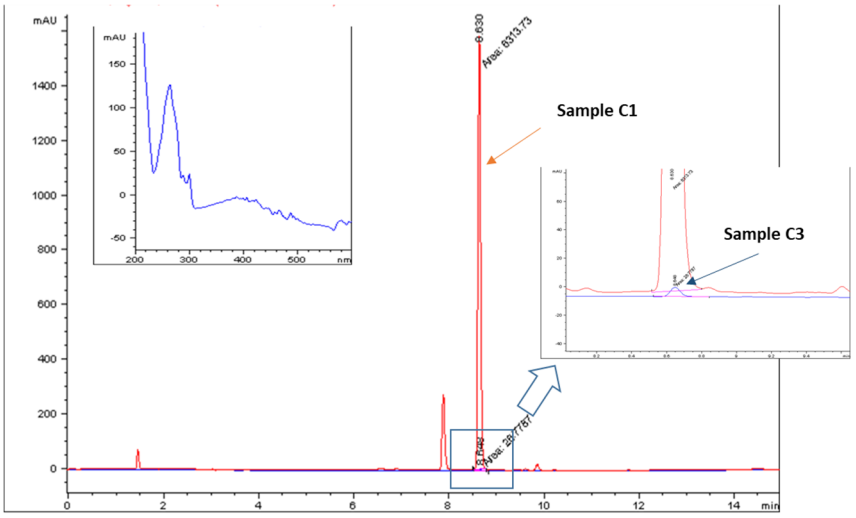
Appendix: Typical Kit Performance Data (LC Analysis, CellMosaic)

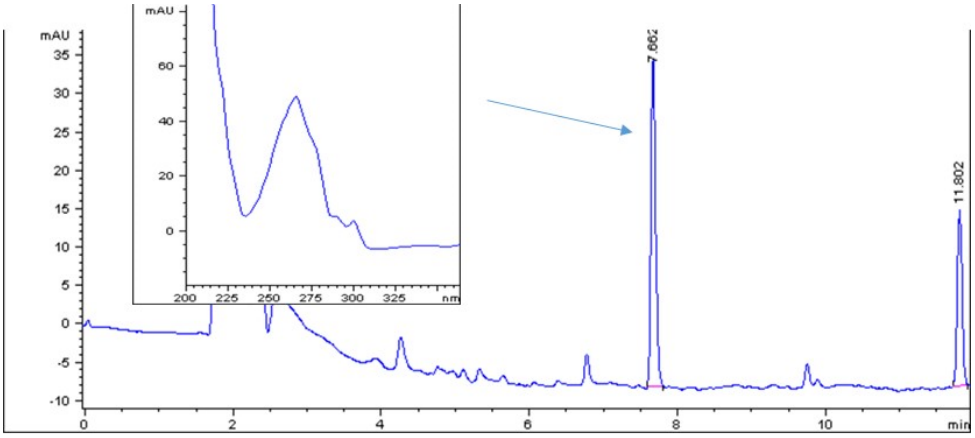
Small molecule information: Fmoc-Gly-OH

Figure 1: C18 HPLC/UV analysis of Fmoc-Gly-OH before (sample **C1**) and after immobilization (sample **C3**) (detected at 254 nm). Left inset: UV spectrum of Fmoc-Gly-OH peak 8.63 min. Right insert: expansion of the HPLC profile from 8.0 to 9.6 min.



Calculate Loading: (6313.73-26.7787)x10/6313.73= 9.96 µmole per mL

Figure 2: C18 HPLC/UV analysis of deprotected resin. Inset: UV spectrum of Fmoc-piperidine adduct at 7.662 min.



Experiment for analyzing the Fmoc group loaded onto the resin: After immobilization of Fmoc-Gly-OH, ~100 µL of beads were removed and added into 200 µL of 20% piperidine in NMP. After mixing for 15 minutes, 5 µL were removed for HPLC analysis. The HPLC profile is identical to the profile obtained from Fmoc-Gly-OH deprotection, confirming that Fmoc-Gly-OH is loaded onto the resin.