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SepSphere™ Small Molecule Amine Immobilization Kit (CM71005) 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 an amine functional group onto Glyoxyl-modified agarose beads.

⚠ Upon receipt, please remove **Box 1** and store in a refrigerator at 2-8°C. Store **Box 2** at room temperature.

	Name	Part #	Quantity	Storage condition
Box 1	Glyoxyl-modified 4% agarose beads	CM71602	5 mL	2-8 °C
	Solution A (**blue label**)	CM01006	1.5 mL	
	Buffer A (reaction buffer, **green label**)	CM02010	20 mL	
	Buffer B (reducing buffer: 4.5M $NaBH_4$ in 2M NaOH) (**red label**)	CM02016	0.6 mL	
	Buffer C (neutralization buffer: 2N HCl) (**cyan label**)	CM02015	0.7 mL	
	Buffer D (washing buffer 1) (**yellow label**)	CM02011	30 mL	
	Buffer E (washing buffer 2) (**orange label**)	CM02012	30 mL	
	Storage buffer (0.02% NaN_3 in PBS) (**grey label**)	CM02008	10 mL	
Box 2	2.0 mL centrifuge tubes	CM03CT3	3	Ambient Temp
	HPLC vials	CM03PT2	2	
	Column Set	CM03SC7	1	
	Stirrer	CM03PT1	4	
	Airtight Syringe 20 mL	CM03SR1	1	
User Material	Small molecule amine	N/A	NOT PROVIDED (10–50 µmol needed)	
	Deionized water	N/A	NOT PROVIDED	

Loading and Small Molecule Amount:

Small molecule immobilization via glyoxyl-activated agarose beads is very efficient and offers very high small molecule loading. CellMosaic's kit can result in a maximum of 90 µmol of small molecule per mL of settle beads based on the *O-(4-Nitrobenzyl) hydroxylamine* immobilization test. If you are targeting maximum loading, you will need ~70 µmol of small molecule assuming 80% of your small molecule is immobilized. This translates to about 35 mg of a 500 Da molecular weight (MW) small molecule.

However, depending on your application requirements for loading and the amount of small molecule you can supply, you can decide how much small molecule you will use for immobilization. The recommended loading target of small molecule for CM71005 is ~1–10 µmol/mL of settled beads for better bead property.

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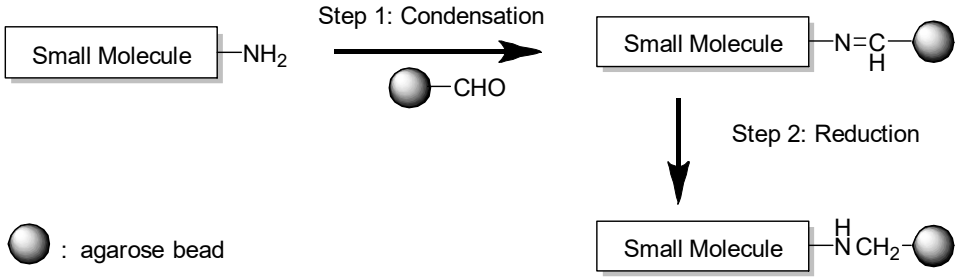
Safety Information

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 amino functional groups. The user supplies the small molecule. Using the kit components, the user immobilizes the small molecule amine onto glyoxyl agarose through reductive amination (**Scheme 1**).



Scheme 1: Immobilization of Small Molecule Amine through Reductive Amination.

Key features of this SepSphere™ immobilization kit:

- Offers a simple and easy way to immobilize small molecules with amino group
- Fast and easy preparation: 5 h preparation and less than 30 minutes hands-on time
- Stable linkage (no release of the small molecules from the resin under acidic or basic conditions)
- All reagents and supplies included for preparation

Requirements for small molecule amine:
1. Preferably > 90% pure
2. Absence of ketone, aldehyde, double bond, or any other functional groups that might be reduced
3. Preferably non-hindered aliphatic primary amine
4. For molecule containing aromatic amine or secondary amine, please consult CellMosaic prior to conducting the experiment.

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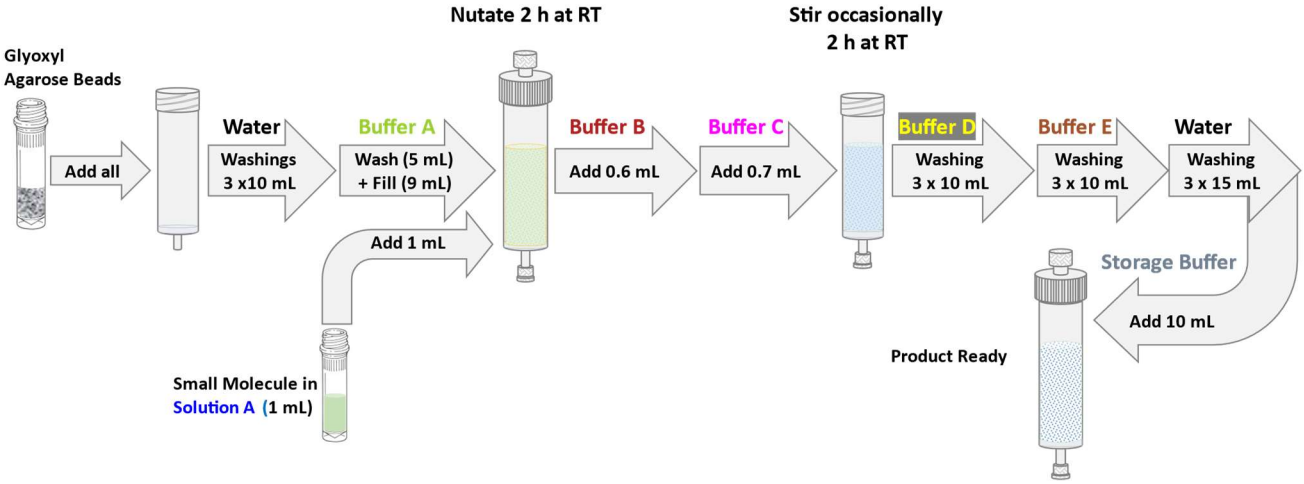
Protocol



Scheme 2: Workflow of Immobilization of Small Molecule Amine.

1. Lab Instrumentation Needed

- Vortex mixer
- Pipettes and tips
- Timer
- Nutating mixer or 3-D Rocker
- Balance
- Erlenmeyer flask

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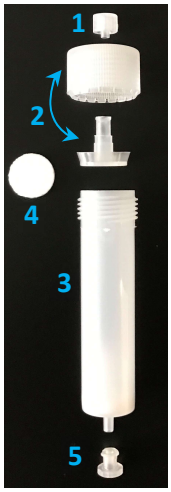
2. Immobilization Lab Techniques at CellMosaic (for reference)

CellMosaic has a universal workflow and kit design that allow immobilization of small molecules and large biomolecules, washing of unreacted starting materials, and column packing all in one column with simple set-up in any lab. The immobilization efficiency in the column is the same as if performed in a regular reaction vessel. The washing is performed with a simple manual air push mechanism using a syringe and requires no gas/air flow or vacuum. The following table outlines the kit component design and key techniques used at CellMosaic for immobilization. Please refer to these key techniques while performing the immobilization according to the user manual.



Column design and setup:

Design: Column comprises five pieces (see left picture)

1. **Male Luer lock cap** (referred to as **top cap** in the protocol and is used during the mixing/nutation).
2. **Column top with Luer attachment** (referred to as **column top** in the protocol). The Luer attachment is for a tight seal and for attachment of the syringe for washing. Column top is removed for addition of buffer and stirring.
3. **Column body containing one polypropylene frit at the bottom** (referred to as **column** in the protocol).
4. **One extra frit for column packing after immobilization** (referred to as **frit** in the protocol).
5. **Bottom female Luer lock plug** (referred to as **bottom plug** and is used during mixing/nutation and storage).

Setup (see right picture): Securely attach the column to a support stand, lab frame, or any support rod and place a beaker or a flask under the column for waste collection.

Use of buffer(s) with resin in column

Design: All of CellMosaic's buffers are supplied in small tubes and are easy to pour directly into the column. Washing buffers generally come with the exact amount needed for optimal washes. The combined wash-volume is fixed, regardless of how much you pour each time.

Procedure:

1. Remove the top cap.
2. Unscrew the column top and bottom plug.
3. Place them on a clean surface for reuse later.
4. Pour 8-10 mL of **any buffer** into the column containing resin for immobilization (~2 volumes of the resin bed).

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Mixing resin in column

Design: The kit comes with one or a few blue polypropylene stirrers of the right length for individual columns. The stirrer is resistant to general solvents, and resin in general will not stick to the stirrer.

Procedure for mixing resin with washing buffer: Mix the resin with buffer using a clean stirrer for ~5-10 seconds. Make sure the stirrer goes into the bottom of the column and the resin is mixed well. Then remove the stirrer without any resin adhering to it. Set aside the stirrer for repeated washing.

Procedure for mixing resin with reaction buffer: Mix the resin with buffer using a clean stirrer for 10-30 seconds. Make sure the stirrer goes into the bottom of the column and the resin is mixed well. Then remove the stirrer without any resin adhering to it. Dispose of the stirrer as solid waste. **Note:** For some reactions, gas/air bubbles may be generated. Stir slowly and continuously until most of the air bubbles are gone before capping it for mixing (nutation).

Washing resin after stirring

Design: The kit comes with one 20 mL airtight syringe with slip tip that can be used to push air through the column.

Procedure for washing:

1. Attach the column top to the Luer attachment after stirring.
2. Draw 20 mL of air into the syringe and attach the syringe to the column top.
3. Push the air through the column.
4. Once the plunger reaches the bottom of the syringe, detach the syringe. Repeat the air drawing and purging process. In general, there will be approximately 1.2 mL of residual liquid after several pushes. It is OK to go on to the next washing step.
5. Remove the column top, fill with buffer, and repeat the washing and purging process.

Procedure to remove residual liquid: If residual liquid is to be removed for certain reactions (see individual protocol), use this additional step after air purging with the syringe. Place the column into a 50 mL centrifuge tube with the column top attached without the top cap. Create a counterbalance with a 50 mL centrifuge tube with water in it. Place both tubes into a quick spin centrifuge, such as IEC clinical centrifuge. Set the spin to the maximum setting and spin for 15-20 seconds. Alternatively, you can use conventional centrifuge equipment and spin at ≤750 x g for 1 minute.

Column setup for immobilization:

After adding reagents per the protocol, attach the column top and securely cap/plug the column top and bottom. Check to make sure everything is tight and there is no leak before putting the column in a nutator. If you do not have a mixer or nutator, leave the column open and stirrer in place. Stir the resin every 5-10 minutes for the specified time.

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Storage buffer and others:

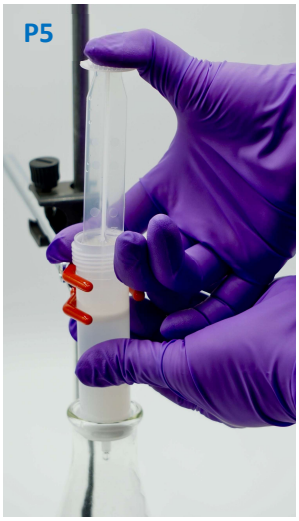
The kit comes with standard storage buffer (1x PBS with 0.02% NaN_3). If you need to use your own buffer, substitute your buffer during the last washing.

Column packing:

Design: You can pack the resin in the column once the immobilization is done. A separate polypropylene frit is included in the kit for column packing. Otherwise, you can store the resin in the column and pipette out as needed for usage.

Procedure:

1. Remove the top cap. Unscrew the column top and bottom plug.
2. Fill the column with deionized water or buffer up to the top and wait a few minutes so that most of the resin settles down.



3. Wet the frit with deionized water before placing the frit inside.
4. Remove the black rubber cap in the plunger of the 20 mL syringe.
5. Use the plunger to push the frit inside the column and all the way down to the top of the resin. Make sure the pressure is even and the frit is flat and not tilted. There should be no air in between the frit and the resin before pushing the frit all the way down to the top of the resin.
6. Once the frit reaches the top of the resin, use slight pressure to make the frit tight.
7. Attach the column top with Luer attachment securely and cap the top.

The column is ready to use. You can use gravity flow or attach the column to a peristatic pump with Luer lock adapters.

Protocol for immobilization starts on next page

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3. Preparation of Agarose Beads

Items needed: Glyoxyl-modified 4% agarose beads (CM71602), Column set (CM03SC7), Airtight syringe 20 mL (CM03SR1), Buffer A (CM02010, green label), Stirrer (CM03PT1), deionized water & a clean flask for collection.

A1. Securely attach the **Column** to a support stand, lab frame, or any support rod and place a flask under the column for collection. Remove the top cap. Unscrew the column top and bottom plug. Place them on a clean surface for reuse later.

A2. Slightly centrifuge the tube containing agarose beads to ensure no bead is in the cap before opening. Pour all the beads into the **Column**, can use some water to transfer.

A3. Add 10 mL of **deionized water** into the column. Stir the resin with a clean stirrer for 5 seconds. Set aside the stirrer for later use.

A4. Attach the column top. Use the 20 mL syringe to push the liquid out.

A5. Remove the column top and repeat the washing (**Steps A3 and A4**) two times (total three washings)

A6. Remove the column top, add 5 mL of **Buffer A** (**Reaction Buffer)** into the column. Stir the resin with a clean stirrer for 5 seconds. Set aside the stirrer for later use.

A7. Attach the column top. Use the 20 mL syringe to push the liquid out.

A8. Securely cap the end of the column. Open the column top and add 9 mL of **Buffer A**.

Glyoxyl Agarose Bead is Ready for Immobilization

4. Immobilization (Scheme 1 & 2)

Items needed: Solution A (CM01006, blue label), Buffer B (CM02016, red label), Buffer C (CM02015, cyan label), Buffer D (CM02011, yellow label), Buffer E (CM02012, orange label), Storage Buffer (CM02008, grey label), 2.0 mL centrifuge tubes (CM03CT3), HPLC vials (CM03PT2), stirrer, Airtight syringe 20 mL (CM03SR1), deionized water & a clean flask for collection.

B1. Weigh 10 to 50 µmol of **Small Molecule (SM)** into a 2.0 mL centrifuge tube and add 1 mL of **Solution A.** Cap and vortex for 30 seconds or sonicate for a few minutes to ensure all of the solid is dissolved. Discard any unused **Solution A** as chemical waste.

Calculation: Amount of SM (mg) = Molecular Weight (MW) of SM x _____µmol

B2. Transfer the Small Molecule solution from **Step B1** into the column containing agarose beads from **Step A8** and securely cap the column.

HPLC Sample B2 (Optional). If you are planning to remove the sample for HPLC analysis, please follow these steps:

- Mix the column content for 30 sec with stirrer.
- Transfer 200 µL of liquid from column into a 1.5 mL centrifuge tube.
- Centrifuge at 14,000 x g for 1 minute.
- Transfer 100 µL of the supernatant to an HPLC vial labeled **Sample B2**.

B3. Nutate the column (end-to-end mixing) at room temperature for 2 h.

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Safe Handling Chemical Notice: Buffer B contains 4.5M $NaBH_4$ in 2M NaOH. **Buffer C** contains 2N HCl. Both are very corrosive hazardous material. Wear proper personal protection equipment (nitrile glove, lab coat, and eye goggle) to handle these chemicals. Read SDS prior the experiment.

B4. In a fume hood, secure the column, open the top cap, and add the whole content of **Buffer B** (0.6 mL of reducing buffer, 4.5M $NaBH_4$ in 2M NaOH) into the column, mix with stirrer.

B5. Add the whole content of **Buffer C** (0.7 mL of neutralization buffer, 2N HCl) drop by drop into the column. Use the stirrer to stir the solution. A lot of gas will be generated during the stirring.

B6. Let the column sit at room temperature with the top open for **2 h**. Use a stirrer supplied to stir the solution occasionally to mix the resin (every 10-15 minutes).

HPLC Sample B6 (Optional). If you are planning to remove the sample for HPLC analysis, please follow these steps:

- Transfer 200 µL of liquid from column into a 1.5 mL centrifuge tube.
- Centrifuge at 14,000 x g for 1 minute.
- Transfer 100 µL of the supernatant to an HPLC vial labeled **Sample B6**.

B7. Attach the column top. Use the 20 mL syringe to push the liquid out.

B8. Open column top, add 10 mL of **Buffer D (Washing buffer 1)**. into the column. Stir the resin with a clean stirrer for 5 seconds. Set aside the stirrer for later use.

B9. Attach the column top. Use the 20 mL syringe to push the liquid out.

B10. Remove the column top and repeat the washing (**Steps B8 and B9**) two times (total 3 washings)

B11. Following similar wash processes, wash the agarose beads in column with 10 mL of **Buffer E (Washing buffer 2)** 3 times and 15 mL of deionized water three times.

B12: For long term storage, transfer the beads to a suitable container (or use the column with caps), add 10 mL of **Storage Buffer** and store it in a 4-8 °C refrigerator.

Small Molecule Conjugated Agarose Bead is Ready for Your Experiment

In general, around 20-80% of the amines will be loaded onto the column. The approximate loading of your small molecule on agarose is 1−10 µmol per mL of settled beads depending on the amount you added.

Specification for your product

Matrix: 4% beaded agarose (source: GE Life Sciences, Sepharose® 4B)
Particle size: 45 – 165 µm
Ligand concentration: 2-6 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 have a HPLC detector that can detect your small molecule (UV-Vis), you can remove two samples and analyze them by HPLC. Alternatively, you can send the samples to CellMosaic for analysis on your behalf (**SKU:AS0021**).

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 B2**]-[HPLC area of **Sample B6** x 1.13]) x 10 /[HPLC area of sample B2]