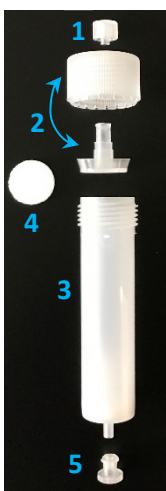


Immobilization Lab Techniques at CellMosaic

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 using a simple manual air push mechanism with 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 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 attachment of the syringe for washing. The 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 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).



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 generally 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. Remove the stirrer without any resin adhering to it and set it aside 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. 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 the column for mixing (nutating).



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 the column 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 containing with water. Place both tubes into a quick spin centrifuge, such as an 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 $\leq 750 \times g$ 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.



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 after the immobilization step is complete. 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 the column.
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 of 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. Securely attach the column top with the Luer attachment and cap the top.



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