

Column Assembly Protocol

See figures at end of protocol for images of column parts and an assembled column.

1. Screw a stopcock onto a barrel (3mL syringe without plunger). Put the stopcock in the closed position (level perpendicular to barrel).
2. Insert a frit into the column barrel and push it down to the bottom of the barrel.
3. Clamp the column onto a ring stand or other 'holder'. Place an empty beaker beneath the column to catch liquid that drips through.
4. Mix up the HIC resin in the bottle until it is homogeneously mixed. The HIC resin comes suspended in 20% ethanol.
5. Measure up 1.5mL of resin solution and dispense it gently into the column. Allow the resin to settle. Once it is packed down, your column should be about 1cm high. You can add more resin if needed.
6. Open the stopcock valve and allow the liquid to begin to drip out. Sometimes it is necessary to apply some pressure to get the liquid flowing. This can be done using a 5mL syringe to push air into the top of the column, or by putting a tissue over the top of the column and blowing hard until drops begin to come out.
7. Close the stopcock valve.
8. Put a tip cap on the bottom end of the column.
9. Put an end cap on the top of the column.
10. You can store the columns like this until you are ready to use them.
11. Before use, allow all of the 20% ethanol to drip out. Clamp the column to a ring stand and place a beaker beneath it.
12. Remove the tip cap and end cap. Open the stopcock valve and allow the liquid to drip through.
13. When there is a small amount of liquid remaining at the top of the column, add 2mL of column equilibration buffer and allow that to drip through.
14. Add another 2mL of column equilibration buffer. You can store the columns like this (remember to close the stopcock valve and put a tip cap and an end cap on). Or you can allow the equilibration buffer to drain through and continue on with RFP or GFP purification.

NOTES:

- When storing or transporting columns keep them upright and immobile if possible. If they bounce around too much or tip over, the resin will flow around in the column barrel and will lose its packed state. If this happens, place the column upright. Remove the end cap and place the stopcock in the open position. Using a 1mL micropipettor, mix up the resin a bit. Then allow the resin to settle so it settles in a properly packed manner. Close the stopcock. Put the end cap back on. The column resin **MUST** be kept moist during use and storage. Make sure there is

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liquid (at least 1 inch) above the resin bed. Make sure the stopcock is in the closed position.
Make sure the tip cap and end cap are both on tightly.

- Columns can be reused multiple times. But you must make sure everything is eluted from the column and that the column is re-equilibrated with Column Equilibration buffer.
- Sometimes resin gets past the frit and leaks out when various buffers are passing over the column. If this happens at a low level don't worry about it. The resin will fall to the bottom of the tube and the eluted protein can easily be removed without getting resin along with it. If this happens at a high level don't use the column. When assembling a column try to select a frit that fits fairly tightly in the column barrel. There seems to be a bit of variation in frit size.

