

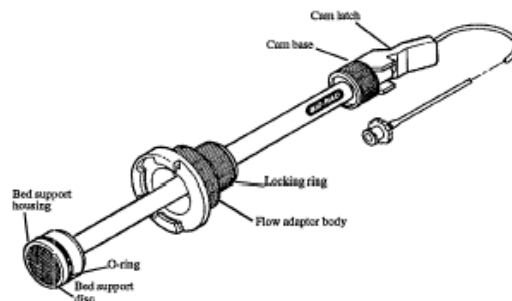


Column Adaptor Instructions



PURPOSE OF THIS DOCUMENT: To instruct the use of the column BioRad column adaptor.

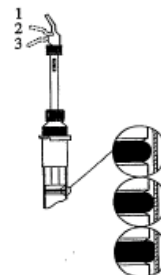
ASSEMBLY AND LOCATION - Each laboratory pair has one labeled 1.0 cm diameter column adaptor assigned to them. The adaptors are to stay on the bench when not in use. The remainders of the column adaptors are in the chromatography section of the biochem lab drawer.



PREPARATION - Check bed support housing for dried material. Examine the bed support housing to ensure that it is properly fitted (screwed into) to the inner grey cam tube. Check the tube for dried material/buffer salt crystals. Look for kinks in tubing that would block buffer flow. Check connections for proper fit. **Be careful when twisting or screwing together the flow adaptor. They are expensive and can snap if more than finger tight pressure is applied!**



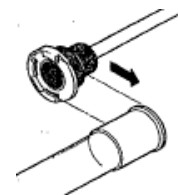
USE - The specific instructions are found in detail in the BioRad manual - sections 2 -6. These should be fully read to understand the use of the flow adaptor. See the pdf file on the class website. Practice with an empty column before packing your column. See the Tegity for a demonstration.



Three positions of flow adaptor cam latch.

Short Instructions.

- There are three fittings for the cam latch loose, medium and tight. Ensure the adaptor is placed into the column when in the loose (#1) setting.
- Pack the column and leave 2-3 cm of buffer above the resin. Flow the buffer of choice through the pump, but leave the flow adaptor hose empty.
- With the **column closed**, slide the flow adaptor body onto the column top **above** the buffer. **The column tubing should be open.**
- Slide the adaptor into the column. If the adaptor is too tight, loosen by turning the cam latch (while holding the white adaptor body AND the cam base). If the adaptor is still tight, try wetting the rubber o-ring.
- While holding the white adaptor body and the cam base, turn the cam latch clockwise until the o-ring just starts to touch the glass, then back the latch $\frac{1}{4}$ turn. The adaptor should still move fairly freely.
- GENTLY slide the column adaptor into the buffer above the resin while still in latch position 1. You must avoid leaving air bubbles between the adaptor and the resin. This can be done by tipping the column to evacuate the bubble or slowly moving the adaptor up and down. Care must be taken to avoid disturbing your packed column.
- Once the adaptor is to the top of the buffer, move the latch to the middle position.





Column Adaptor Instructions



- Slowly lower the adaptor to just above the top of the resin. As you move the adaptor to the resin, the buffer should come out through the column adaptor tubing. If buffer does not come out through the tubing, and instead flows around the adaptor o-ring, the latch is too loose. Back out the adaptor, adjust the cam latch tighter and repeat. **Failure to ensure the buffer comes out of the tube will result in air from the tube being forced through the column.**
- Move the cam latch to position 3. The o-ring should be firmly seated against the glass. Turn the cam latch $\frac{1}{4}$ to $\frac{1}{2}$ turn tighter. Tighten the locking ring to secure the adaptor.
- Flow your buffer through the column adaptor and column, only after you are certain there is no remaining air. Don't forget to open the column only after the pump is attached or the tubing leading to the adaptor/column is in a beaker with buffer.
- ALWAYS check the adaptor to see that the buffer is going through the column and not out and around the top of the adaptor.
- If the resin compresses, re-adjust the flow adaptor with the column stopped and the flow adaptor in the number 2 position (the adaptor's tubing should be disconnected from the pump).

TROUBLE SHOOTING - Most of the problems encountered are due to the flow adaptor being too tight or loose in the number 3 position or with air in the tube before running the column.

CLEANING AND STORAGE - After each use, the tubing must be flushed with water and disconnected from the pump. Leaving buffer in the tubing will result in salt crystals.