

Make a protein resin using CNBr-activated Sepharose 4B

This protocol can be used to make antibody resin or any other protein you need to couple to a resin. M1 anti-FLAG antibody is a good example

1. measure protein yield with nanodrop.
2. The resin binds around 10 mg/ml of antibody so calculate how much resin you need
3. **Bring resin to RT before opening the container!**
4. Add cold 1 mM HCl (36% HCl is 11.65 M so it is 86 μ l per 1 L) to the dry resin to make it swell. The ratio is 3.5 times swelling.
5. Follow the [manufacturer's protocol](#) to bind resin, typically incubate O/N while rotating at 4 °C - this can be done in a capped column.
6. Load resin on column and let binding reaction flow through. Collect the followthrough and measure protein content, which should be close to zero.
7. Block column with 5% milk or other blocking reagent.
8. Wash column with storage buffer (HBS).
9. Add azide (2 mM) to protect from contamination.

Store resin in PBS or HBS with 2 mM sodium azide. With proper handling it will work for at least ten uses, potentially much more. The most important thing is to wash with acid after each use, and then neutralize promptly. I also filter all dirty samples prior to loading, which helps immensely in avoiding long-term slow clogging with repeated use.