PURIFICATION OF FUSION PROTEIN ON IgG SEPHAROSE 6 FF

1. Pack the column with 4 ml of IgG Sepharose 6 FF suspension to obtain a 2 ml column.
2. Wash the column with 5 bed volumes of Tx-Swb prior to use in order to remove traces of EtOH.
3. Equilibriate the column with 2-3 bed volumes each of:
    1. 0.5M HAc, pH 3.4
    2. Tx-Swb
    3. 0.5M HAc, pH 3.4
    4. Tx-Swb

    Check the pH of the eluate with pH paper and adjust pH of sample is necessary (both should be neutral). Apply the sample to the column.
4. Wash the gel with:
    1. 2 bed volumes of Tx-Swb
    2. 5 bed volumes of Tris-NaCl (0.1M) *
    3. 2 bed volumes of 5 mM NH4Ac, pH 3.4
5. Elute the sample with 0.5M HAc, pH 3.4
6. Collect the first 15ml as eluate.
7. Re-equilibrate IgG Sepharose 6 FF with 5-10 bed volumes of Tx-Swb until effluent is above pH 7.0.

Note: Wash off Triton, because it absorbs in the UV range. When reusing gel after this point, it requires washing with 50ml Tx-Swb before washing with Tris-NaCl beginning at step #4.

Tx-Swb:

- 1% Triton X-100
- 100mM Tris pH 8.0
- 10mm EDTA
- 1mm PM/SF
- 100mM NaCl