Antibody purification
(Harlowe and Lane, Antibodies: A laboratory manual)

1) Wash the column with 10 bed volumes of 10mM Tris pH 7.5
2) Wash the column with 10 bed volumes of 100mM glycine pH 2.5
3) Wash the column with 10 bed volumes of 10mM Tris pH 8.8
4) Make sure pH of last wash is 8.8. If not, continue to wash
5) Wash the column with 10mM Tris pH 7.5 until the pH of the eluate is 7.5
6) Dilute antibody: 2ml antibody, 8ml of 10mM Tris pH 7.5
7) Pass antibody over column three times

During the next two steps prepare the dialysis tubing
8) Wash the column+antibody with 20 bed volumes 10mM Tris pH 7.5
9) Wash the column+antibody with 20 bed volumes 500mM NaCl, 10mM Tris pH 7.5
10) Elute the antibody with 10 bed volumes of 100mM glycine pH 2.5
    Collect the eluate in a tube with 1 bed volume of 1M Tris pH 8.0 to raise the pH of the antibody immediately. In this protocol the only elution is to break the acid-sensitive interactions. This will be the majority of the interactions caused by the H-bonding during the antibody-peptide interactions (as opposed to the base-sensitive interactions)
11) Wash the column with 10mM Tris pH 7.5 until pH is 7.5. This is just to neutralize the column for future use
12) Store the column with buffer and .02% NaAzide
13) Dialyze the antibody against 1x PBS in 2L for 2hrs
    To prepare dialysis tubing:
    a. Put on gloves.
    b. Cut off a length of dialysis tubing that is longer than you think you will need.
    c. Wet the dialysis tubing under the distilled water faucet. Once the tubing gets wet, keep it wet. Crinkle up the tubing under the running water. Rinse out the inside - there is glycerol inside and this needs to be washed away. Continue this washing for 5 - 10 minutes.
    d. Tie a knot near one end of the tubing. Tie another. Check for leakage. If any solution leaks through these knots, tie another.
    e. Boil for 10 minutes in a large volume of 2% NaHCO3 and 1mM EDTA pH8.0
    f. Always keep the tubing submerged and don’t touch it with hands
    g. Before use, wash the tubing inside and out with distilled water
    h. Immerse the knotted tubing in dialysis buffer (keep a tub near side of sink).
    This will be whatever buffer your protocol calls for. Keep the tubing in the buffer until you are ready to load your sample.
14) Then 2L overnight or another 2 hours
15) Add .02% NaAzide (and 25ul NGS/ml)
20) Proceed to concentration protocol or aliquot into smaller volumes