Purification Of Anti Peptide Antibody

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Protocol:

All steps up to the dialysis at rt.

1. Pour column in TBS (=0.15M NaCl, 20mM TrisCl pH 7.4). We use a 5 ml column for 25 mls serum. Wash extensively in TBS after prewashing as indicated in the protocol for coupling peptide to the resin.
2. Thaw serum- dilute 1:1 with TBS and filter through a 0.2 um filter
3. Load the serum over the column, taking at least 20 minute total.
4. Run the breakthrough over the column five times. Alternatively you can use a parastaltic pump and recirculate the serum ON or just batch bind the serum ON.
5. Wash with 5 col vols TBS.
6. Wash with 10 col vols 0.5 M NaCl, 20mM TrisCl pH 7.4, 0.2% Triton-X-100.
7. Wash 5 col vols TBS
8. Elute with 0.15 M NaCl, 0.2 M Glycine-HCl pH 2.0. Collect 1 ml fraction, with each tube containing 0.1ml of 2 M TrisCl pH 8.5
9. Wash with TBS until pH is reequillibrated.
10. Elute with 6 M GuanidineHCl in TBS, collecting 1ml fractions.
11. Wash with TBS + 0.1% NaN3, and store at 4 deg C.
12. To determine where to pool fractions, spot 1 ul of each fraction onto nitrocellulose paper and stain with ponceau S. Pool all fractions that show pink color.
13. Dialyze ON into TBS or your favorite buffer.
14. If necessary the antibody can be concentrated by sweating the dialysis bags, or by spin-concentrating.
15. Bring the azide concentration up to 0.1% and store at 4 deg C for up to three months. For longer storage freeze in aliquots and store at -80 degC or add glycerol to 50% and store at -20 deg C.

Note: Do not pool the low pH and GuHCl eluates as they may have significantly different properties. We have found that the GuHCl may

have higher affinities, but also may contain a higher fraction of partially denatured antibody that could contribute to staining background. The proportion in each pool varies with the peptide immunogen.

The quality of the anti-peptide serum seems to increase with multiple boosts - the first bleed may be feeble.