Protocol for Production of Anti-Mouse Patched Antibody.

Vectors:

1. PGex:mPtc-Cterm (Amp resistant). Ptc C-terminus (aa~1238-1413) fused at its N-terminus to GST. Used for injection into animals.
2. pMal:mPtc-Cterm (Amp resistant). Ptc C-terminus (aa~1205-1434) fused at N-terminus to MBP. Used for affinity purification.

Preparing Antigen for Injecting Rabbits:

1. Freshly transform Vector#1 above in to BL21 cells (for all our protein production, we use cells from Stratagene cat#230245).
2. Protocol for growth and induction is identical to that supplied by Amersham/GE (the manufacturer of the PGex plasmid series). Briefly cells are grown to an OD600 of 0.4 AU and induced with 0.25mM IPTG for 3-4 hours, spun down, washed in PBS and frozen at -80 until protein purification.
3. Lyse cells in 10 pellet volumes of lysis buffer (PBS, 1% Triton X-100, 1mM PMSF, 0.5mgs/ml lysozyme, 1X complete PI tablets (Roche), 1mM DTT). Rotate at 4 degrees for 30 min and then sonicate to shear DNA.
4. Spin (20000xg for 1 hour) and save supernatant.
5. Incubate supernatant with glutathione sepharose (GE/Amersham) to capture the GST-fusion protein. Wash beads with 20-30 column volumes of lysis buffer.
6. Elute protein from the GST column with standard 2X SDS-PAGE Sample Buffer. Run on preparative 8% polyacrylamide gels, cut out band and inject into Rabbits (we used Josman Labs LLC in Napa).
7. We routinely run analytical SDS PAGE gels to monitor the protein at various steps and to estimate quantity by comparison to a BSA standard.

Preparation of Affinity Purification Resin:

1. Freshly transform Vector#2 into BL21 Cells.
2. Grow and induce as described above for the pGex plasmid.
3. Lysis buffer is 1X PBS, 1mM PMSF, 0.25% Triton X-100, 0.5mgs/ml lysozyme, 1X complete PI tablets (from Roche), 0.5mM DTT. Sonicate to shear DNA and complete lysis of cells. Clarify lysate by centrifugation 20000xg for 1 hour, save supernatant.
4. Capture the MBP fusion protein by incubating the lysate with Amylose resin (New England Biolabs). Wash resin with 20-30 column volumes of 1X PBS+0.5mM DTT.
5. Elute MBP fusion protein from amylose with 1X PBS+0.2mMDTT+ 10mM Maltose. Dialyze protein into PBS.
4. Couple the protein covalently to Aminolink Sepharose (Pierce) using instructions of the manufacturer.
5. Use this resin for affinity purification of antibodies from immunized rabbit serum. We use the protocol exactly as described in Harlow and Lane “Using Antibodies” for affinity purification.