Purification of T Cell Receptor

Protein Harvests:

David grew all the cells and did all of the harvesting on the Cell Pharm for this protein. I filtered the harvests through a 0.45 µm filter, and added azide to 0.05% and EDTA to 1 mM final concentration. I pooled the harvests into roughly 3 L batches for purification on antibody affinity columns.

Purification:

This protocol came from Roland (he got it from the Davis lab at Stanford). Both the KJ25 (anti-β chain) and the A2B4 (anti-α chain) columns were made by Roland. Flow the combined harvest over the KJ25 column, wash with 5-10 column volumes PBS / 0.05% azide / 1 mM EDTA, and elute with 40 ml 100mM acetate / 1 M NaCl / 10% glycerol / 0.05% azide (pH 4.0). Neutralize the eluate with 4 ml 1 M Tris, pH 7.4, and check the A280 to see if there is any protein in the eluate. If there is protein, run the eluate over the A2B4 column, wash with 5-10 column volumes PBS / 0.05% azide / 1 mM EDTA, and elute with 100 mM citrate / 1 M NaCl / 10% glycerol / 0.05% azide / 5 mM EDTA. Collect 1 ml fractions; put 100 µl 1 M Tris, pH 7.4 in the collection tubes to neutralize the acid. Concentrate any fractions containing protein and dialyze them into PBS / 0.05% azide / 1 mM EDTA. Re-equilibrate the two columns with PBS / 0.05% azide / 1 mM EDTA, and run more protein sample over them.

Note: I have never seen an appreciable amount of protein coming off of these columns. I still have some doubt whether or not the affinity columns actually work, since I have never been able to purify any protein from the Cell Pharm harvests. However, immunoprecipitations done by David on the harvests have shown protein.