UK stably. Using the Biosilon microcarrier in our culture system, and the CD-1 cell line as a model, pro-UK was stably secreted for several weeks, and its production could be enhanced along with increasing cell density. We believe that cell density and pro-UK yield can be enhanced further when culture conditions are optimized.

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A New Method For Purification of Anti-DNA Antibodies

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The purification of anti-DNA antibody is a critical step in studying the structure and function of the antibody. Here a new method for the purification of anti-DNA antibody by affinity chromatography is detailed. This method is also suitable for purification of DNA-binding proteins (Nelson WJ, et al. Biochem Biophys Res Comun, 1982, 106:1141).

A DNA-cellulose matrix was prepared as follows: 1 g of calf thymus DNA was dissolved in 100 ml of H_2O and then heated to 100℃ for 10 min to achieve DNA denaturation. The ssDNA solution was cooled down rapidly by pouring it into liquid nitrogen, then placed in ice, and subsequently mixed with 50 g of CF-11 cellulose powder (Pharmacia) that had been reduced with $NaBH_4$ in a 60℃ water bath for 1 h. The ssDNA cellulose slurry was lyophilized and forced through a fine sieve. One gram of the fine powder was suspended in 50 ml of absolute ethanol. To immobilize ssDNA on the cellulose, the suspension was UV-irradiated under an Original Hanau Q81 lamp (Hanau, FRG) for 1 min in an ice bath, followed by washing with 500 ml of 0.1 mol/L NaOH and then with 1 L of H_2O, and finally lyophilized. The matrix is about 10 mg ssDNA/g cellulose (approximately 50% coupling of the input DNA).

A glass column was filled with the prepared matrix and then equilibrated with the buffer containing 300 mmol/L NaCl, 20 mmol/L NaH_2PO_4, pH 7.2. In order to avoid nonspecific proteins binding to the ssDNA-cellulose, 300 mmol/L NaCl was added to the hybridoma culture supernatant. The supernatant with high ionic strength was applied onto the ssDNA column. Flow rate was adjusted at 30 ml/h. After washing the column with the equilibrate buffer, bound antibodies were eluted with 6 mol/L guanidine-HCl. The eluant was extensively dialyzed against a buffer with 150 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 8.0. Every second fraction was detected by SDS-PAGE, immunoblot and ELISA. The results showed that the eluant contained pure antibodies and displayed reactivity with ssDNA, while the fractions that flowed through the column did not. This method was simple, efficient and had a good yield.