Purification Of A Human Monoclonal IgM Antibody From Bioreactor Supernatant Using A Combination Of Cation And Anion Exchange Chromatography

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Abstract

Two protocols are currently available for the purification of monoclonal antibodies (mAbs). Here, we describe a novel method for the purification of a recombinant monoclonal antibody, SK-1.45, produced by a hybridoma cell line in a bioreactor. Using protein-free tissue culture media, we have developed an automated process for the purification of the antibody. The process involves a step procedure involving cation and anion exchange chromatography on Sephadex® G-25 (Pharmacia) and Macro-Prep® high Q support (Bio-Rad Laboratories). Partially fractionated using cation exchange chromatography, SK-1.45 is produced by a hybridoma cell line in a bioreactor using protein-free tissue culture media. They contain no protein. With GMP requirements and cost restraints associated with purification, yields and poor immunoreactivity of the recovered products have been a problem. Several methods have been employed to purify SK-1.45, with specificity for an adenocarcinoma associated antigen. SK-1.45 is produced by a hybridoma cell line in a bioreactor using protein-free tissue culture media. They contain no protein.

Introduction

For more than 15 years, hybridoma technology has provided access to antibody products of well defined, homogeneous quality. Antibody products are being used as biologics in the biomedical research industry. They also have potential as diagnostic reagents, pharmaceuticals (biologically active and disease-specific antigens), vaccines, and in the production of therapeutics and immunotherapeutics. Antibody products can be produced using recombinant DNA technology in either mammalian or bacterial systems. A number of factors influence the success of current purification methods, including antibody performance, specificity, and cost. While some factors are directly related to the antibody used for purification, other factors are related to the purification method used. The combination of cation and anion exchange chromatography is one such method. This method allows for the purification of antibodies in a single-step process.

Procedure Overview

For SK-1.45, this procedure resulted in a final product that was highly homogeneous and had a > 98% purity as determined by SDS-PAGE. This product was produced using a combination of cation and anion exchange chromatography.

Buffer Exchange

Cation Exchange Chromatography

Anion Exchange Chromatography

In Process Material

Purified SK-1.45

Results

Yield = IgM recovered from starting material.
Purity = Determined with SDS-PAGE.

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Purification of a human monoclonal IgM antibody from bioreactor supernatant using a combination of cation and anion exchange chromatography.