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PURIFICATION AND IODINATION OF MONOCLONAL ANTIBODIES FOR THE MEASUREMENT OF HUMAN PROINSULIN BY TWO-SITE IMMUNORADIOMETRIC ASSAY. Haber EP, Tarumoto C, Xu D, Borghi VC, Wajchenberg BL. Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP) and Laboratório de Investigação Médica (LIM-25) da Faculdade de Medicina da Universidade de São Paulo, Brazil.

The problem of measuring insulin specifically in human serum is compounded by the presence of both intact proinsulin and partially processed proinsulin. Assays capable of specifically measuring the wide range of insulin-like molecules in serum has been achieved by the use of a panel of monoclonal antibodies (mAcs) raised against these molecules. This work describes the isolation and radioiodination of specific monoclonal antibodies to be used in a two-site immunoradiometric assay for measurement of intact proinsulin concentrations. Ascitic fluid of Balb/c mice containing mAcs against human biosyntethic proinsulin (Eli Lilly Co., USA) were purified by precipitation with caprilic acid. The immunoglobulin fraction was dialysed against phosphate buffered saline (PBS) and then further purified by addition of ammonium sulphate. After dialysis against PBS, the protein concentration of the precipitate was determined by absorbance of UV irradiation (A_{280} nm). The antibodies were shown to be pure by electrophoresis on a 7% polyacrylamide gel. Purified monoclonal antibodies (20 µg) were iodinated by the Iodogen method using 1 mCi of NaI-125. Unreacted iodide was separated on a 1 x 25 cm column of Sephadex G25 eluted with 0.05 M sodium phosphate buffer, pH 7.4, containing 1% bovine serum albumin (BSA) and thiomersal. The specific activity of the antibodies was 20 µCi/µg. The peak containing the labeled antibody was stored at 4 °C until assayed.

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