

CONJUGATION OF ANTI-IMMUNOGLOBULIN (M), ANTI IGMFLUOROCHROME DERIVATIVE (FITC, ISMOER 1)

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ABSTRACT

Immunochemical methods, based on antigen-antibody interaction, have become the most important-tool in qualitative and quantitative plasma proteins determinations due to these high specifically and sensitivity. Immunoglobuline can be divided into five classes IgG, IgA, IgM, IgE and IgD. The normal human serum IgM is about 0.5-2.0 mg/ml. IgM is the dominant antibody formed early in the primary immune response to most antigens (immunogens). Its concentration in the circulation is high enough for it to be purified from normal serum. However, the preparation is facilitated by the use of serum from patients with waldenstromsmacroglobulinaemia. Because of the large size (Mwt. 900,000) for IgM, gel filtration is an important fractionation step in the preparation. The chromatographically purified IgM fraction antiserum is conjugated with Fluorescein-Isothiocyanate (FITC, Isomer 1). After conjugation, removing of unreacted (unconjugated) and reacted (conjugated) fluorochrome is carried out using gel filtration. The conjugation of anti-IgM-FITC proceeded by nucleophile attack of the unprotonated ϵ - amino group of lysine to the fluorochrome, resulting in a thiourea bond in alkaline medium.

KEYWORDS: Immunoglobulin (M), Anti-IgM, Fluorochrome Derivative, IgA Immunofluoresene