PRODUCT SPECIFICATION

HSR 008 1st SSI Purified MBL Standard
From human plasma

Article No. 71801
Product Name HSR 008 1st SSI Purified MBL Standard
Description Preparation: 350 μl affinity purified MBL, sterile filtered.
Content: 192.6 μg/mL (range 156.0-229.2) pure Mannan-Binding Lectin (MBL) in complex with MASPs, ratio 76:24.
Solvent: TBS, pH 7.4, with 0.5% mannose + 2% maltose added as stabilizing agents.
Storage: -20 ºC
Exp. date: 16 weeks from shipment date (see label)

Background
MBL is a C-type lectin and an important component in innate immunity. MBL is an oligomer i.e. forming dimers to hexamers of homotrimeric subunits of ~26 kDa polypeptides. This oligomerisation is essential for functional activity.

MBL forms a non-covalent complex with specific MBL-associated serine proteases (MASPs), termed MASP-1, -2, and -3. Upon binding to the surface of a pathogen MASP-activation is initiated with subsequent complement activation and clearance through lysis or phagocytosis1.

MBL-deficiency is the most common immune defect resulting in susceptibility to severe infections in early childhood or if immuno-suppressed2. MBL-deficiency has also been associated with several clinical disorders, e.g. autoimmune diseases, endocarditis, and septicaemia2,3. Normal levels of oligomeric MBL in serum are 1 – 5 μg/mL whereas MBL-deficient serum levels are < 100 ng/mL, when estimated by a standard ELISA for MBL quantification.

Preparation
An MBL-rich fraction from human plasma (tested negative for HbsAg, antibodies to HIV-1/2 and HCV) was purified by affinity chromatography, ion-exchange chromatography and gel-filtration. Virus reduction by S/D treatment and nano-filtration4. Further purified by immuno-affinity chromatography to remove IgA, IgM and alpha2-macro-globulin, and finally carbohydrate-affinity chromatography5.

MBL concentration was determined by performing total amino acid analysis on a Biochrom 30 analyzer and correcting the total protein concentration found with the ratio of MBL:MASPs determined by densitometric scanning of the bands in lane 2 of the SDS gel shown below5.

Application
The concentration of HSR 008 (192.6 μg/ml (range 156-229.2 μg/ml)) was determined by catching ELISA5. Hence, HSR 008 is wellsuited for catching ELISA with an antibody directed against MBL as catching antibody. HSR 008 is not applicable for functional ELISAs e.g. with mannan-binding.

Analysing HSR 008 by SDS-PAGE (red. conditions) (fig 1) results in a dominant band at ~32 kDa (monomeric MBL), and some weak bands deriving from MASP-1, -2 and -3 as well as fragments of these5.

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Fig 1: Separation of protein standard (left lane) and HSR 008 1st SSI Purified MBL Standard (right lane, loaded with 1.4 μg MBL) on a 4–20% Mini-PROTEAN® TGX Stain-Free™ Protein Gel. A Gel Doc EZ Imager was used for fluorescent detection of the protein bands.

NB: Please be aware that the concentration ranges shown above are for total MBL— if measuring functional MBL the levels will be reduced significantly due to partial inactivation during the purification steps.

HSR 008 should not be stored in smaller aliquots than 350 μl. HSR 008 may be stored at +5°C for no more than 14 days or 16 weeks at -20°C. HSR 008 is stable for at least two freeze-thaw cycles.

Reference


Conditions

For research use only. Not for use in diagnostic procedures. Not for therapeutic use. This product may not be resold, modified for resale or used to manufacture commercial products (including as part of a kit) without prior written approval from Statens Serum Institut.

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