Human MBL Oligomer ELISA

For the quantitation of human MBL concentrations in serum, and plasma.

INTRODUCTION

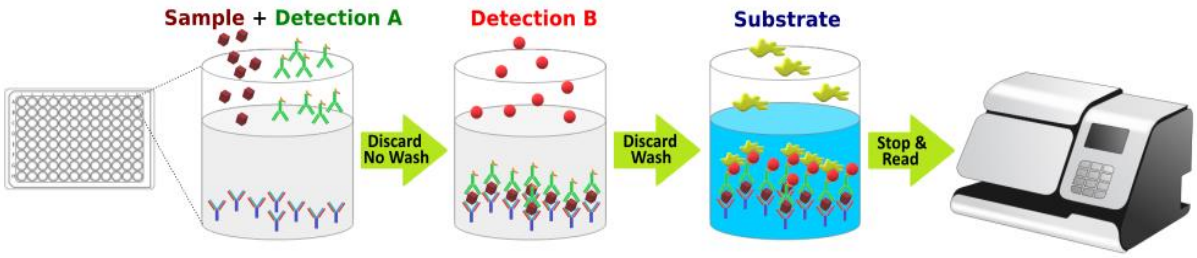
Mannan-binding lectin (MBL), also called mannose binding lectin or protein (MBP), is a multimeric carbohydrate-binding protein produced in the liver and secreted into the blood. It is an important component in innate immune system. Although MBL has multiple forms, only the normally oligomerized forms of MBL are functional, i.e. capable of binding efficiently to carbohydrates and associating with the MASPs. The MBL level can be used as a diagnostic indicator for MBL-associated disease.

The Tribio® Human MBL ELISA is a solid phase ELISA designed to measure human MBL levels in serum and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detection into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 2 hours, not need 5-6 hours (Fig. 1). The measurement can be finished within 2 hours, not need 4-5 hours. The detection range is from 0.125 to 20 ng/mL. The levels of human MBL samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human MBL protein levels.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique. A MBL antibody was pre-coated onto a microplate. Standards or samples are pipetted into the wells, and then, incubated with biotin-conjugated detection antibody specific for human MBL. Then, just aspirate each well, no wash, and directly add horseradish peroxidase (HRP)-conjugated streptavidin, and incubation. Followed by washing to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of MBL bound in the initial step, which measured by plate read at 450 nm.

Fig. 1 Assay Principle



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE CONDITIONS
Human MBL Microplate	TBS32105A	96 well strip microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human MBL.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human MBL Standard (Lyophilized)	TBS32105B	20ng Recombinant human MBL protein. Reconstitute in 0.5mL DD-water	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32105C	2.2 ml of Biotin-Human MBL antibody.	May be stored for up to1 months at 2-8 °C.
Detection B	TBS32105D	300 µL HRP-Streptavidin (1:50 dilution).	
Assay Diluent	TBS32105E	12 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

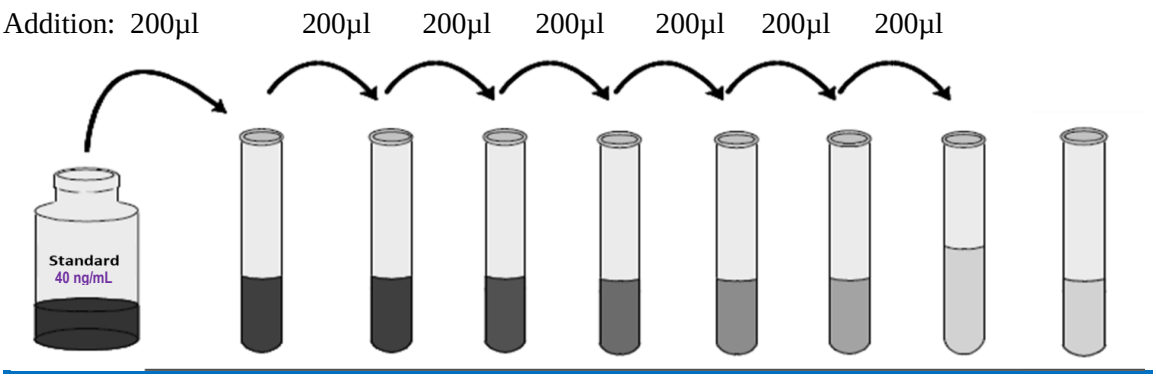
Detection B (freshly prepared before use): Add 250 µL of Detection B Concentrate (1:50) to Assay Diluent to prepare 10 mL of Detection B solution.

Human MBL Standard reconstitution: Reconstitute lyophilized Standard (20 ng) by adding 0.5 mL Assay Diluent to make 40ng/mL standard stock.

Human MBL Standard Preparation: Label test tubes as #1 through #8. Pipet 200 µL of 1x Assay Diluent into tube #1 to #8 **as diagram below.**

1. Add 200 µL of the Human MBL Standard stock solution (40 ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(20 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human MBL standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	200	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	200	200	200	200	200	200	200	0
Final Conc (pg/mL)	20000	10000	5000	2500	1250	625	312.5	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours.**
3. Aspirate each well. Invert the plate and blot it against clean paper towels (***no wash***).
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 1 hour.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light***)**. The color becomes blue**.** If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human MBL concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is provided for demonstration only as Fig.3. A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The minimum detectable dose (MOD) of human MBL is typically 100 pg/ml.

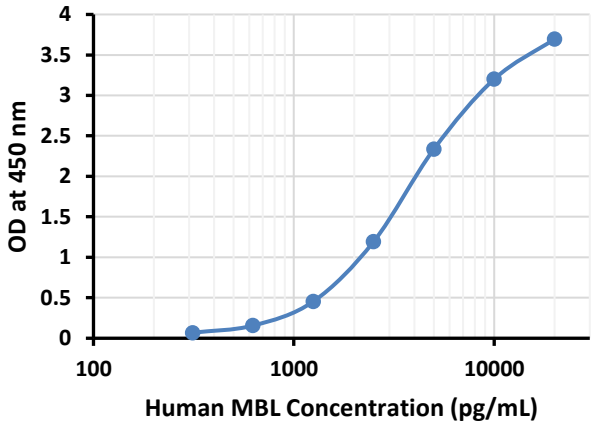
SPECIFICITY

This assay recognizes natural and recombinant human MBL.

RELATIVE PRODUCTS

Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (TBS5001)
Protein Assay Kit (TBS2005)
TMB Substrate System (TBS5021)

Fig.3 Human MBL Oligomer ELISA



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