MBL Oligomer
ELISA Kit

KIT 029RUO

REF Σ 96

For Research Use Only.
Not for use in diagnostic procedures.

invitrogen
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Please read these instructions carefully

APPLICATION

For the in-vitro determination of oligomerized mannan-binding lectin in human serum or heparin plasma. For research use only. Not for use in diagnostic procedures.

INTRODUCTION

Mannan-binding lectin (MBL; also called mannose-binding lectin or protein) is a multimeric carbohydrate-binding protein produced in the liver and secreted into the blood, where it constitutes an important element in innate immune defense against invading microorganisms. Its normally oligomerized forms are associated with specfic serine proproteases (the MASPs) which are activated when MBL binds to microbial carbohydrate surfaces and in turn activate complement via the MBL or lectin pathway.

PRINCIPLE OF THE ASSAY PROCEDURE

The assay is an ELISA performed in microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain. Bound MBL is detected with the same antibody that has been labeled with biotin, followed by development with horseradish peroxidase (HRP)-conjugated strept-avidin and incubation with a chromogenic substrate. Comparison of the assay results with molecular size chromato graphy of MBL immunoreactivity in individual human serum samples suggests that the monoclonal antibody used is selective for MBL oligomers when used as both capture and detection antibody. The assay is a four-step procedure:

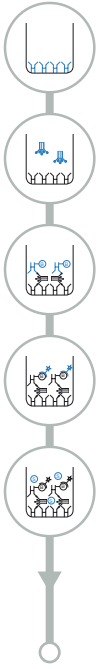
Step 1. Aliquots of calibrators, diluted serum samples and any controls are incubated in micro wells precoated with monoclonal antibody against MBL. MBL present in the solutions will bind to the antibody-coated wells via its carbohydrate-binding domains. Unbound material is removed by washing.

Step 2. Biotinylated monoclonal detection antibody is added to each test well and incubated. The detection antibody attaches to bound MBL oligomers via carbohydrate-binding domains that are not occupied by being bound down to the coat. Unbound detection antibody is removed by washing.

Step 3. HRP-conjugated streptavidin is added to each test well and allowed to form a complex with the bound biotinylated antibody. Unbound conjugate is removed by washing.

Step 4. A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test well. The bound HRP-streptavidin reacts with the substrate to generate a colored product. The enzymatic reaction is stopped chemically, and the color intensity is read at 450 nm in an ELISA reader. The color intensity (optical density) is a function of the concentration of MBL oligomeric forms originally added to each well. The results for the calibrators are used to construct a calibration curve from which the concentrations of MBL in the test specimens are read.

PRINCIPLE OF THE ASSAY PROCEDURE



MBL Antibody
Plates are precoated with MBL antibody. The plates are ready for use

MBL
Diluted samples and calibrators are added to each well and incubated

Biotinylated MBL Antibody
Biotinylated detection antibody is added to each well and incubated

Streptavidin – HRP
HRP-conjugated streptavidin is added to each well and incubated

TMB Substrate
Substrate is added to each well. Develop for 15 minutes in the dark

Stop Solution
Stop Solution is added to each well. Read plate within 30 min.

Quantitative results are obtained by measuring the absorbances of the wells at 450 nm

KIT COMPONENTS

Item	Contents	Quantity
1	12 x 8 coated Microwells + Frame	96 wells
2	Sample Diluent	1 x 60 mL
3	MBL Calibrator 1-8 0, 0.5, 1, 2, 10, 20, 40 ng/mL	8 x 1 mL
4	25x Wash Solution Conc.	1 x 30 mL
5	Biotinylated MBL Antibody	1 x 12 mL
6	HRP-Streptavidin	1 x 12 mL
7	TMB Substrate	1 x 12 mL
8	Stop Solution	1 x 16 mL

Note: Liquid reagents contain preservatives and may be harmful if ingested.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Adjustable micropipettes covering the range 1-1000 µL and corresponding disposable pipette tips
2. Polypropylene tubes to contain up to 1000 µL
3. Tube racks
4. Adjustable 8- or 12-channel micropipette (50-250 µL range) or repeating micropipette (optional).
5. Clean 1 L graduated cylinder
6. Deionized or distilled water
7. Cover for microplate
8. Clean container for diluted Wash Solution
9. Apparatus for filling wells during washing procedure (optional)
10. Lint-free paper towels or absorbent paper
11. Disposable pipetting reservoirs
12. Timer (60-minute range)
13. Calibrated ELISA plate reader capable of reading at 450 nm (preferably subtracting reference values at 650 or 620 nm)

14. Sodium hypochlorite (household bleach 1:10 dilution) for decontamination of specimens, reagents, and materials

PRECAUTIONS

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1. This kit should only be used by qualified laboratory staff.
2. The MBL calibrators were prepared from MBL purified from human plasma. Each blood unit used for its preparation was tested by approved methods and found to be nonreactive for hepatitis B surface antigen (HBsAg) and antibodies against human immunodeficiency virus (HIV 1 and 2, and hepatitis C virus (HCV). In addition, the product was subjected to virus deactivation procedures. However, as no test method can offer complete security that infectious agents are absent, the calibrators and specimens should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety In Microbiology and Biomedical Laboratories", 1999. Solutions containing human serum should be treated as potentially infectious and handled accordingly.
3. Use separate pipette tips for each sample, calibrator and reagent to avoid cross-contamination.
4. Use separate reservoirs for each reagent. This applies especially to the TMB Substrate.
5. After use, decontaminate all specimens, reagents and materials by soaking for at least 30 minutes in sodium hypochlorite solution (household bleach diluted 1:10).
6. To avoid droplet formation during washing, aspirate the wash solution into a bottle containing bleach.
7. Avoid release to the environment. Dispose of containers and unused contents in a safe way and in accordance with national and local regulations.
8. Reagents in this kit contain preservatives and may be toxic if ingested.
9. The Stop Solution contains 0.5 mol/L sulfuric acid and can cause irritation or burns to the skin and eyes. If contact occurs, rinse immediately with plenty of water and seek medical advice.
10. Do not interchange components from kits with different batch numbers. The components have been standardized as a unit for a given batch.
11. Hemolyzed or hyperlipemic specimens may give erroneous results.
12. Do not dilute serum or plasma specimens directly in the microwells.
13. Do not touch or scrape the bottom of the microwells when pipetting or aspirating fluid.
14. Incubation times and temperatures other than those specified may give erroneous results.
15. Do not allow the wells to dry once the assay has begun.
16. The TMB Substrate is light sensitive. Keep away from bright light.
17. Do not reuse microwells or pour reagents back into their bottles once dispensed.
18. Wear gloves when handling specimen during collection of specimen, preparation of specimen or procedure.
19. The device contains material of human or animal origin and should be handled as potential carrier and transmitter of disease. The bovine serum albumin is manufactured from USDA-inspected bovine plasma and is validated for prion (TSE) clearance.
20. Do not use the kit if the packaging is damaged.

STABILITY AND STORAGE

1. Store the kit with all reagents at 2-8°C. Do not freeze.
2. Use all reagents before the expiry date on the vial labels.
3. Diluted Wash Solution remains stable for 4 weeks at 2-8°C. If not all wells are to be used, dilute only the portion of Wash Solution Concentrate required.

4. For subsequent use, store unused wells in the foil pouch with the desiccant provided and reseal. Always allow foil pouch to equilibrate to room temperature before opening to avoid condensation in/on the coated microwells.

COLLECTION OF SPECIMENS

Handle and dispose of all blood, serum or plasma specimens as if they were potentially infectious. See Precautions, sections 2, 3, 5, 6, 7 and 18.

Determination of MBL in a single specimen requires 5-15 µL of serum or plasma. Blood specimens should be collected aseptically into a plain or heparinized tube by qualified staff using approved venipuncture techniques. Serum or plasma should be prepared by standard techniques for clinical laboratory testing. Cap the specimens and store them at 2-8°C for assay within 24 hours. If the assay cannot be performed within 24 hours or if the specimen is to be shipped, cap the specimen and keep it frozen at –20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens.

PREPARATION OF REAGENTS AND SAMPLES

1. Bring all specimens and reagents to room temperature (20-25°C). Mix specimens thoroughly by gentle inversion and if necessary clear visible particulate matter by low-speed centrifugation.
2. Determine the number of specimens to be tested (in duplicate) plus any internal laboratory control specimens (in duplicate) plus any reagent blank wells. The pre-coated wells can be used as strips of 8 or as individual wells. Single wells are handled by breaking individual wells apart and placing each well in the frame at an appropriate position. Add 16 wells for the 8 calibrators (in duplicate). Remove the number of microwells required and replace the remainder in the foil pouch with desiccant at 2-8°C.
3. Wash Solution: Dilute the 25x Wash Solution Concentrate by pouring the total contents of the bottle (30 mL) into a 1-L graduated cylinder and add distilled or deionized water to a final volume of 750 mL. Mix thoroughly and store at 2-8°C after use.
4. Sample Diluent: Ready to use, do not dilute further.
5. MBL Calibrators: Ready to use. The assigned concentrations are indicated on their labels. Do not dilute further.
6. Biotinylated MBL Antibody: Ready to use, do not dilute further.
7. HRP-Streptavidin Conjugate: Ready to use, do not dilute further.
8. TMB Substrate: Ready to use, do not dilute further.
9. Stop Solution: Ready to use, do not dilute further.
10. Specimens: Dilute each specimen in a recorded proportion with Sample Diluent to obtain at least 250 µL of diluted solution that can be set up in duplicate wells at 100 µL per well. An initial screening at a dilution of 1/100 (e.g. 5 µL of serum + 495 µL of Sample Diluent, mixed by inversion or slow vortexing) is suggested for most samples, followed by re-assay of out-of-range samples at lower or higher dilution, as appropriate. Dilutions lower than 1/20 should not be used.

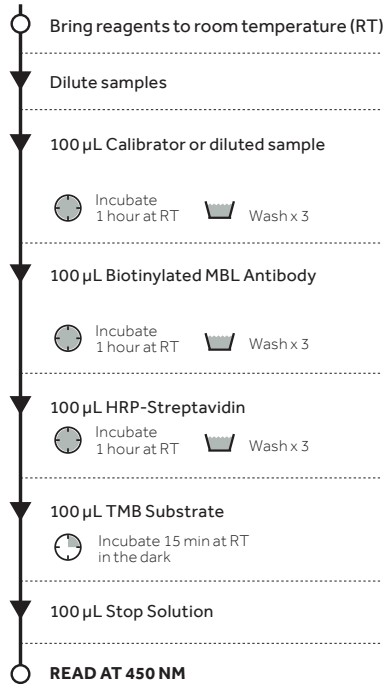
ASSAY PROCEDURE

1. Prepare the assay protocol, assigning the appropriate wells for setting up calibrators, diluted specimens and any internal laboratory controls in duplicate. If a reference wavelength of 650 or 620 nm is not available on the ELISA reader, a reagent blank well can be assigned. This is set up with 100 µL of Sample Diluent instead of diluted serum or plasma and processed like the other wells.
2. Pipette 100 µL volumes of each calibrator, diluted specimens and any internal laboratory controls into the corresponding positions in the microw-

ells. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform set at 200/minute.

3. Aspirate the contents of the microwells and wash the microwells three times with at least 300 µL of diluted Wash Solution. If washing is done manually, empty the microwells by inversion and gentle shaking into a suitable container, followed by blotting in the inverted position on a paper towel. A dwell time of 1 minute before emptying is recommended for at least the last wash of the cycle. The vigor with which Wash Solution is filled into or emptied from the wells influences final color development. Manual pipetting, which may be very gentle and lead to high color development, is only recommended in the absence of alternatives such as filling the wells by immersion, using a multi-channel manual washing dispenser, or using an automatic washing apparatus.
4. Dispense 100 µL of Biotinylated MBL Antibody (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
5. Wash as described above in Step 3.
6. Dispense 100 µL of HRP-Streptavidin Conjugate (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
7. Wash as described above in Step 3.
8. Dispense 100 µL of TMB Substrate (ready to use) into each microwell. The use of a multichannel micropipette is recommended to reduce pipetting time. Cover the wells and incubate for exactly 15 minutes at room temperature in the dark. Start the clock when filling the first well.
9. Add 100 µL of Stop Solution (ready to use) to each well, maintaining the same pipetting sequence and rate as in Step 8. Mix by gentle shaking for 20 seconds, avoiding splashing. Read the wells within 30 minutes.
10. Read the absorbances of the wells at 450 nm in an appropriate microplate reader (reference wavelength 650 or 620 nm). If no reference wavelength is available, the value of the reagent blank well is subtracted from each of the other values before other calculations are performed.

SCHEMATIC OVERVIEW



CALCULATION OF RESULTS

A calibration curve is constructed by plotting the mean of duplicate absorbance values for each MBL Calibrator on the y-axis against the corresponding MBL concentrations in ng/mL on the x-axis. The calibration curve must meet the validation requirements. The MBL concentration of each diluted serum sample is then found by locating the point on the curve corresponding to the mean of duplicate absorbance values for the diluted serum sample and reading its corresponding concentration in ng/mL from the x axis. The concentration of MBL in the undiluted serum specimen is calculated by multiply-ing this result by the sample dilution factor.

This procedure can be performed manually using graph paper with linear x and y scales. A smooth curve can be drawn through the points or adjacent points can be joined by straight lines. The latter procedure may slightly overestimate concentration values between points when the curve is slightly convex to left, which is the typical finding. Although the curve may approximate to a straight line, it is both practically and theoretically incorrect to calculate and draw the straight line of best fit and to read the results from this.

The procedure can also be performed by an ELISA reader software program incorporating curve fitting procedures. The procedure of choice is to use linear x and y axes with 4-parameter logistic curve fitting.

Diluted samples that give a mean absorbance above that for the 40 ng/mL MBL Calibrator or below that for the 0.5 ng/mL MBL Calibrator are out of the range of the assay and their concentrations should be noted as >40 ng/mL and <0.5 ng/mL respectively. The corresponding concentrations in the undiluted sera are calculated >(40 x dilution factor) ng/mL and <(0.5 x dilution factor) ng/mL, respectively. These samples should be re-assayed at higher and lower dilutions for high- and low-reading samples, respectively. The new dilution factors should be those estimated to give absorbance values that fall well within the range of the calibration curve, but dilutions lower than 1/20 should not be used.

VALIDATION OF CALIBRATION CURVE

The mean absorbance for the 40 ng/mL MBL Calibrator should be >1.5. The mean absorbance for any MBL Calibrator should be higher than that for the previous MBL Calibrator, e.g. absorbance(10 ng/mL MBL) > absorbance(5 ng/mL). The curve should be slightly convex to the left when the results are plotted on linear axes.

CALIBRATION TROUBLESHOOTING

Out-of-line points for individual calibrators: One or more individual calibrators may give anomalous absorbance readings. One or both of the duplicate values may be out of line, and the mean of the duplicates may be out of line. This error is significant if it impairs satisfactory curve fitting by the 4-parameter logistic method, which as a result of the anomalous value, is shifted away from other calibrator points that are in fact correct. The calibrator points and fitted curve should always be examined for correct fit before any calculations of concentration from it are accepted. A poorly fitting curve will also be revealed by a high value for the sum of residual squares. If only one calibrator is affected, which is not the highest calibrator, two courses of action are possible:

i) An erroneous singlet or duplicate result should be eliminated from the curve, and the remaining results refitted by the 4-parameter logistic procedure. If a satisfactory fit is obtained, provisional concentration results can be calculated from it.

ii) If no satisfactory fit can be obtained in this way, but the curve is otherwise consistent, provisional results can be obtained from straight lines between the means of duplicates, omitting the erroneous point.

If two or more calibrators are affected, the assay should be repeated.

A deviant result for an individual calibrator can be due to operator error or to calibrator deterioration.

If both duplicate values are consistently out of line in successive assays, the calibrator is faulty and should be omitted.

TRACEABILITY OF CALIBRATOR VALUE

Assignment of the MBL value was carried out by ELISA ensuring traceability to in-house standards at Statens Serum Institut (Denmark).

QUALITY CONTROL

Laboratories intending to perform repeated assays should establish their own high-reading (>1000 ng/mL) and low-reading (<100 ng/mL) control sera, stored in small (e.g. 50 µL) aliquots at -20°C or below. An aliquot of each should be thawed and tested in each assay and a record kept of successive results. This serves as a control of test performance, test integrity and operator reliability. The results should be examined for drift (tendency for successive results to rise or fall) or significant deviation from the mean of previous results. Values not deviating by more than 20% from the mean of previous values can be taken to indicate acceptability of the assay. Aliquots of control serum should not be refrozen for repeated assay once thawed, and if a further assay is performed, fresh control aliquots and fresh dilutions of specimens should be used.

LIABILITY

This ELISA Kit is only intended for the in vitro determination of MBL in human serum or heparin plasma. The ELISA Kit is only intended for use by qualified personnel carrying out research activities. If the recipient of this test passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at recipient's own risk secure in favor of ThermoFisher Scientific all limitations of liability herein.

Catalogue number

Batch code


Consult instructions for use


Use by


Manufacturer


Keep away from sunlight

Temperature limitation


Do not reuse


Caution, consult accompanying documents


Biological risk


Do not use if package is damaged

Maximum numbers of tests

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