



**HK327**

**HUMAN  
MBL/MASP-2 ASSAY**

**ELISA KIT**

**PRODUCT INFORMATION & MANUAL**

Read carefully prior to starting procedures!

**ATTENTION**

For use in laboratory research only

Not for clinical or diagnostic use

Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the certificate of quality control for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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## 1. INTENDED USE

The human functional MBL/MASP-2 assay ELISA kit is to be used for the *in vitro* quantitative determination of functional human MBL/MASP-2 in serum, plasma and cell culture supernatant samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The analysis should be performed by trained laboratory professionals.

## 2. INTRODUCTION

The human functional MBL/MASP-2 assay has been developed for the quantitative measurement of active human MBL in serum, plasma and culture medium, through the measurement of the ability of MBL/MASP complexes to initiate C4 cleavage when it is bound to mannan. The assay is based on the assumption of the presence of an excess of MASP-2. Since MASP-2 deficiency is very rare this assumption is justifiable.

The mannan-binding lectin (MBL) pathway of complement activation has been established as the third pathway of complement activation. MBL is a carbohydrate-binding serum protein, which circulates in complex with serine proteases known as mannan-binding lectin associated serine proteases (MASPs). When bound to micro-organisms, the MBL complex activates the complement components C4 and C2, thereby generating the C3 convertase and leading to opsonisation by the deposition of C4b and C3b fragments. MBL is able to activate the complement activation pathway independent of the classical and alternative pathways and plays an important role in innate immunity. In this assay any influence of the classical pathway has been eliminated by using a special MBL-binding buffer, which inhibits the binding of C1q to immune complexes and disrupt the C1 complex, while leaving the carbohydrate binding activity of MBL and the integrity of the MBL complex intact.

Normal human plasma contains an MBL concentration ranging from 3.3 to 1650 U/ml. Up to 12 % of healthy Caucasian blood donors have MBL levels below 33 U/ml. The MBL level correlates with the amount of C4b-depositing capacity. However, the C4b-deposition capacity can vary threefold between individuals with similar MBL concentrations.

The human functional MBL/MASP-2 assay is useful in evaluating the importance of the MBL pathway in relation to clinical manifestations. Several investigations have reported that low levels of MBL correlate with susceptibility to various infections and diseases.

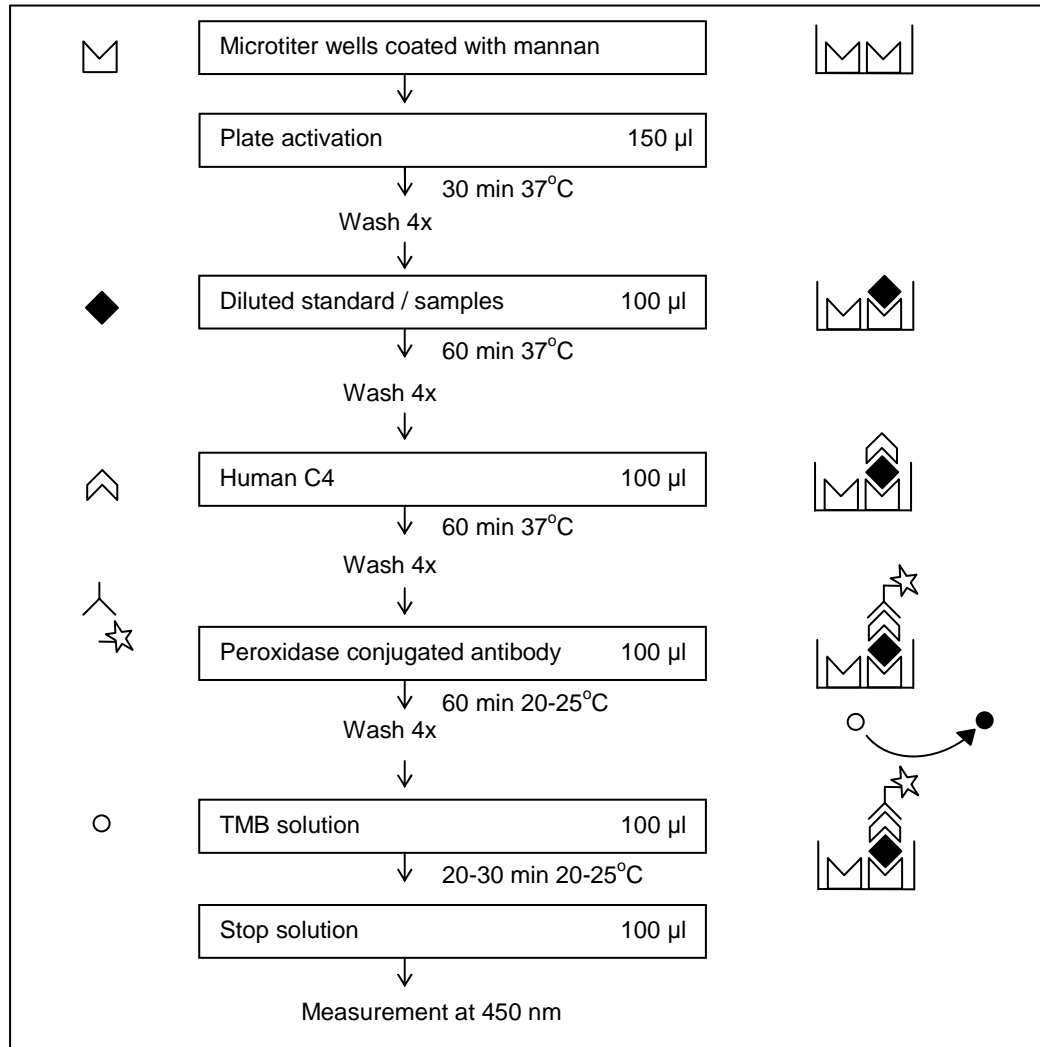
## 3. KIT FEATURES

- Working time of 4 hours.
- Minimum concentration which can be measured is 0.7 U/ml.
- Measurable concentration range of 0.7 to 15 U/ml.
- Working volume of 100 µl/well.

### **Cross-reactivity**

Cross-reactivity for other species or proteins/peptides has not been tested.

#### 4. PROTOCOL OVERVIEW



- The human functional MBL/MASP-2 assay is a ready-to-use ligand-lectin-activation solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 4 hours.
- The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay.
- After activation samples and standards are captured by solid bound mannan. Unbound material present in the sample is removed by washing.
- Human C4 is added to the wells. The C4 binds to the mannan bound functional MBL/MASP-2 complex and can be cleaved.
- Peroxidase conjugated antibody binds to a C4 degradation product bound to the functional MBL/MASP-2 complex.
- Peroxidase conjugated antibody reacts with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the standards (log).
- The human functional MBL/MASP-2 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

## 5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Item no.	Kit component	Quantity	Color code
Vial 1	Plate activation buffer 5x	1 vial (6 ml)	Grey
Vial 2	Wash buffer 40x	1 vial (20 ml)	Grey
Vial 3	MBL-binding buffer 1x	2 vials (20 ml)	Gold
Vial 4	Dilution buffer 10x	1 vial (10 ml)	Gold
Vial 5	Standard	1 vial, 1 ml lyophilized	Yellow
Vial 6	Human C4	2 vials, 1 ml lyophilized	Green
Vial 7	Tracer, peroxidase-labeled	1 vial, 1 ml lyophilized	Blue
Vial 8	TMB substrate	1 vial (20 ml)	Purple
Vial 9	Stop solution	1 vial (20 ml)	Red
Item 10	12 Microtiter strips, pre-coated	2 plates	
Item 11	Frame	1	
Item 12	Adhesive covers	4	
Item 13	Certificate of quality control	1	
Item 14	Manual	1	
Item 15	Data collection sheet	1	

Table 1

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- Standard, Human C4 and conjugate are stable in lyophilized form until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact concentration of the standard is indicated on the label of the vial and the certificate of quality control.
- Once reconstituted, standard, human C4 and conjugate are stable for 1 month if stored at 2 - 8°C.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed until expiration date if stored at 2 - 8°C.

### Materials required but not provided

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.  
In case a plate washer is used the supplied wash buffer is not sufficient. Additional wash buffer can be ordered separately. Please contact your local distributor.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.

## 6. WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Standard, tracer and streptavidin-peroxidase vials should be opened after reconstitution. Open vials carefully: vials are under vacuum.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- The standard is of human origin. It was tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

## **7. SAMPLE PREPARATION**

### **Collection and handling**

#### **Serum or plasma**

Collect blood using normal aseptic techniques. If serum is used, separate serum from blood after clotting at room temperature within 1 hour by centrifugation (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.

If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.

Most reliable results are obtained if EDTA plasma is used.

#### **Storage**

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human functional MBL/MASP-2. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human functional MBL/MASP-2 activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

### **Dilution procedures**

#### **Serum or plasma samples**

Human functional MBL/MASP-2 can be measured accurately if serum or plasma samples are diluted at least 25x with supplied MBL binding buffer in polypropylene tubes.

Note that most reliable results are obtained with EDTA plasma.

#### **Remark regarding recommended sample dilution**

The recommended dilution for samples should be used as a guideline. The recovery of human functional MBL/MASP-2 from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human functional MBL/MASP-2.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

## 8. REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

### Plate activation buffer

Prepare plate activation buffer by mixing 6 ml of 5x plate activation buffer with 24 ml of distilled or de-ionized water, which is enough for 2 x 96 tests. In case less tests are required, prepare the desired volume by diluting 1 part 5x plate activation buffer with 4 parts of distilled or de-ionized water.

### Wash buffer

Prepare wash buffer by mixing 20 ml of 40x wash buffer with 780 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 40x wash buffer with 39 parts of distilled or de-ionized water.

### Dilution buffer

Prepare dilution buffer by mixing 10 ml of the 10x dilution buffer with 90 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

### Standard solution

The standard is reconstituted by injection of 1.0 ml distilled or de-ionized water. Prepare each human functional MBL/MASP-2 standard in polypropylene tubes by serial dilution of the standard with MBL binding buffer as shown in Table 2.

Tube	Volume MBL binding buffer	Volume standard	Concentration (U/ml)
1	See certificate of quality control	150 µl vial 5	15
2	240 µl	360 µl tube 1	9
3	240 µl	360 µl tube 2	5.4
4	240 µl	360 µl tube 3	3.2
5	240 µl	360 µl tube 4	1.9
6	240 µl	360 µl tube 5	1.2
7	240 µl	360 µl tube 6	0.7
8	240 µl	-	0

Table 2

### Human C4 solution

Human C4 is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml human C4 with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. Where less volume is required, prepare the desired volume of human C4 by diluting 1 part of the reconstituted human C4 with 11 parts of dilution buffer.

### Conjugate solution

The conjugate is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml conjugate with 23 ml dilution buffer, which is sufficient for 2 x 96 tests. Where less volume is desired, prepare the required volume of conjugate solution by diluting 1 part of the reconstituted conjugate with 23 parts of dilution buffer.

## 9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 150 µl of diluted plate activation buffer to the assigned wells. Do not touch the side or bottom of the wells.
3. Apply an adhesive cover to the tray. Gently tap the tray to eliminate any air bubbles trapped in the wells. Be careful not to splash liquid onto the cover.
4. Incubate the tray for 30 minutes at 37°C
5. Wash the plates 4 times with wash buffer using a plate washer or as follows:
  - a. Carefully remove the plate sealer, avoid splashing.
  - b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
  - c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
  - d. Repeat the washing procedure 5b/5c three times.
  - e. Empty the plate and gently tap on thick layer of tissues.
6. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
7. Apply an adhesive cover to the tray. Tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
8. Incubate the strips or plate for 1 hour at 37°C.
9. Repeat the wash procedure described in step 5.
10. Add 100 µl of diluted C4 to each well using the same pipetting order as applied in step 6. Do not touch the side or bottom of the wells.
11. Cover the tray with an adhesive cover. Incubate the tray for at 1 hour at 37°C.
12. Repeat the wash procedure described in step 5.
13. Add 100 µl of diluted tracer to each well, using the same pipetting order as applied in step 6. Do not touch the side or bottom of the wells.
14. Cover the tray with an adhesive cover, incubate the tray for 1 hour at room temperature.
15. Repeat the wash procedure described in step 5.
16. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 6. Do not touch the side or bottom of the wells.
17. Cover the tray with a new adhesive cover, incubate the tray for 10 – 30 minutes at room temperature. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
18. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 16. Gently tap the tray to mix the solution and to eliminate air bubbles trapped in the wells.
19. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instruments manufacturer.

## 10. INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale). For an example of the standard curve see certificate of quality control included with the kit. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

## 11. TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- Each time the kit is used, fresh dilutions of standard, sample, tracer, streptavidin-peroxidase and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of supplied covers during incubation steps is necessary.
- The waste disposal should be performed according to your laboratory regulations.

### Technical support

Do not hesitate to contact our technical support team at [support@hycultbiotech.com](mailto:support@hycultbiotech.com) for inquiries and technical support regarding the human functional MBL/MASP-2 assay ELISA.

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## 12. QUALITY CONTROL

The certificate of quality control included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the certificate of quality control are to be used as a guideline only. The results obtained by your laboratory may differ.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Hycult Biotech immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

## 13. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data should be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 3 can be used as a guideline in the case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents were brought to room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colorless
•	•				Wrong filter in the microtiter reader
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions

Table 3

## 14. REFERENCES

1. Petersen, S et al; An assay for the mannan-binding lectin pathway of complement activation. *J Imm Methods* 2001, 257: 107
2. Hamad, I et al; Complement activation by PEGylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover. *Mol Imm* 2008, 45: 3797