EndoCab® IgM

HK504-IgM

Edition 12-12

ELISA KIT
PRODUCT INFORMATION & MANUAL

Read carefully prior to starting procedures!
For use in laboratory research only
Not for clinical or diagnostic use



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info@hycultbiotech.com ● www.hycultbiotech.com

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 Analysis 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 EndoCab® IgM ELISA kit is to be used for the *in vitro* quantitative determination of endotoxin-core antibodies in serum, plasma and cell culture supernatant.

The EndoCab® IgM ELISA kit can be used for different species, like human, rhesus monkey, rat and mouse. However, when using other samples than human, the standard and tracer have to be replaced by a pool plasma of the used species and the right corresponding HRP-labeled antibody.

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 EndoCab® IgM ELISA has been developed for determination of endotoxin core antibodies in human plasma or serum in patients or healthy individuals. Several studies show a consistent drop in postoperative levels of circulating anti-endotoxin core antibodies compared to the preoperative value. This drop has been interpreted as consumption of antibodies to endotoxin by systemic release of endotoxin. A hypothesis is that if the patients pre-operative endotoxin-core level is low, even moderately low, patients may not be able to cope with the efflux of endotoxin, which may have mild to severe clinical consequences. The assay is of interest for various experimental conditions ranging from *in vitro* LPS neutralization by plasma components to *in vivo* studies on kinetics of antibodies to endotoxin in health and diseases.

The EndoCab® standard median-units IgM (MU) are arbitrary and are based on medians of ranges for 1000 healthy adults in a particular locality. It has not been established whether normal endotoxin-core antibody values vary by region, culture or race. Users should establish appropriate local statistical controls for their studies. It is advised that studies of any patient group should always be controled by studies of appropriately selected contrasting clinical groups and/or healthy individuals recruited locally.

EndoCab® is a registered trademark. Used under license from the Scottish National Blood Transfusion Service.

3. KIT FEATURES

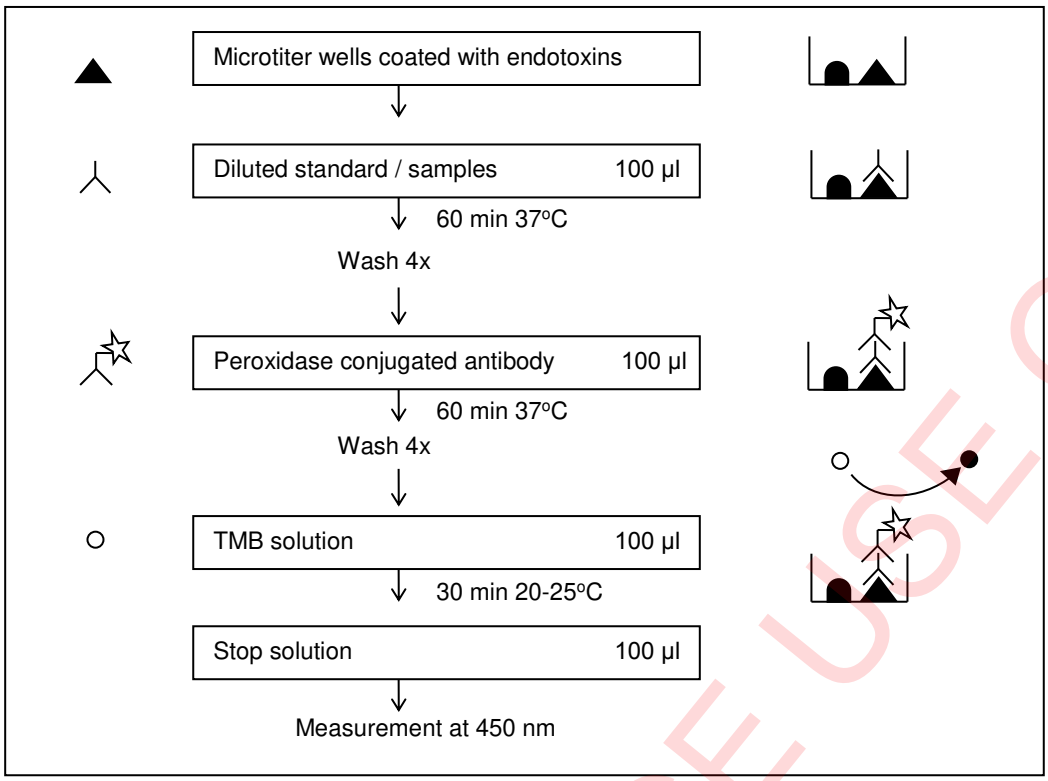
- Working time of 2½ hours.
- IgM endotoxin-core antibodies: minimum concentration which can be measured is 0.05 MMU/ml.
- Measurable IgM concentration range of 0.05 – 3.5 MMU/ml.
- Working volume of 100 µl/well.

Cross –reactivity

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

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4. PROTOCOL OVERVIEW



- The EndoCab® IgM ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 2½ hours.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with equimolar amounts of endotoxin rough-lipopolysaccharides from four Gram-negative bacterial species, each comprised of a complete inner core, but lacking complete outer core or O-specific polysaccharide chain.
- Anti-endotoxin-core antibodies are captured by the solid phase antigen.
- Peroxidase conjugated anti-human IgM antibody will bind to captured endotoxin-core antibodies.
- The peroxidase conjugated antibody will react 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 Endocab® IgM standards (log).
- The endotoxin-core concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

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5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Cat.#	Quantity	Color code
Wash buffer 20x	WB23	1 vial (30 ml)	Colorless
Dilution buffer A 5x	DB86	1 vial (20 ml)	Green
Dilution buffer B 10x	DB87	1 vial (10 ml)	Green
Standard IgM		2 vials, lyophilized	Blue
IgM conjugate, peroxidase-labeled 100x		1 vial (0.120 ml)	Colorless
TMB substrate	TMB050	1 vial (11 ml)	Brown
Stop solution	STOP110	1 vial (22 ml)	Red
12 Microtiter strips, pre-coated		1 plate	
Certificate of Analysis		1	
Manual		1	
Data collection sheet		2	

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.
- The standard in lyophilized form and the conjugate in concentrated solution are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of each standard is indicated on the label of the vial and the Certificate of Analysis.
- After reconstitution the standard is single use and cannot be stored.
- The conjugate can only be stored in concentrated solution and is not stable when stored diluted.
- 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 for 1 month 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.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers can be ordered separately. Please contact your local distributor.
- Centrifuge for 1 ml tubes.

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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.
- Open vials carefully: vials are under vacuum.
- It is advised to spin down conjugate tubes before use.
- 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 advise.
- 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.

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7. SAMPLE PREPARATION

Collection and handling

Serum or plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. 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.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of endotoxin-core antibodies. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of endotoxin-core antibody 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

Endotoxin-core antibodies can be measured accurately if serum or plasma samples are diluted with supplied dilution buffer in polypropylene tubes. Dilute at least 50x for IgM endotoxin-core antibodies.

Note that most reliable results are obtained with EDTA plasma.

Remark regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of endotoxin-core antibodies 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 endotoxin-core antibodies.

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

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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.

Wash buffer

Prepare wash buffer by mixing 30 ml of 20x wash buffer with 570 ml of distilled or de-ionized water, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or de-ionized water.

Dilution buffer

Prepare dilution buffer by mixing 20 ml of the 5x dilution buffer A with 70 ml of distilled or de-ionized water. After homogenizing the solution, add 10 ml of 10x concentrated dilution buffer B, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 2 parts of the 5x dilution buffer A with 7 parts of distilled or de-ionized water plus 1 part of 10x concentrated dilution B. Concentrated dilution buffer may contain crystals. Where 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 pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 1.Prepare each EndoCab® IgM standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*. After reconstitution the standard cannot be stored for repeated use.

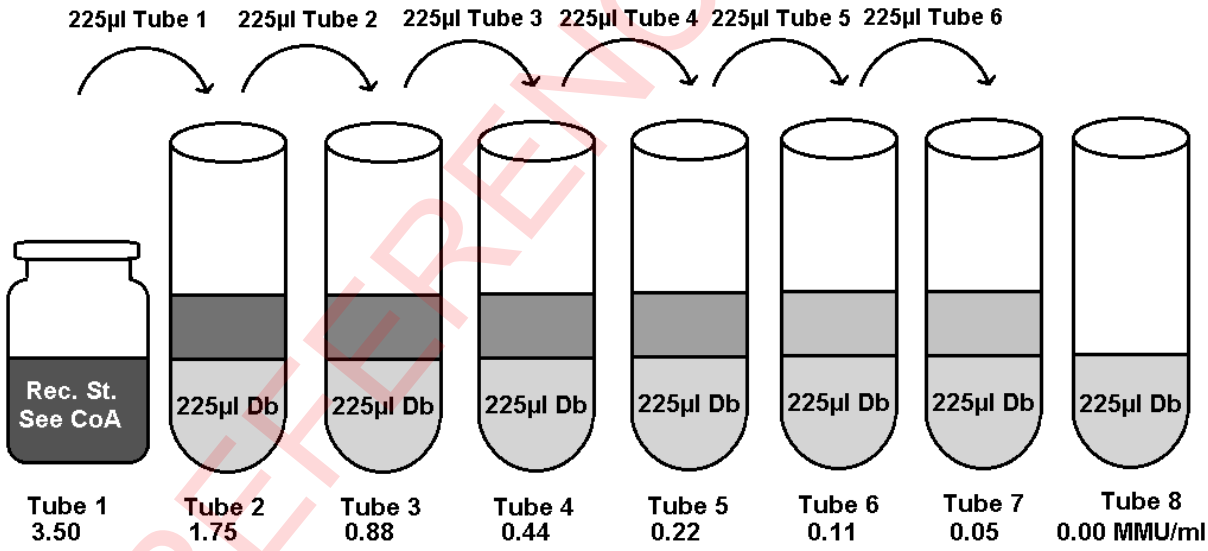


Figure 1

*) CoA: Certificate of Analysis, Rec. St.: Reconstituted Standard, Db: Dilution buffer

Conjugate

It is advised to spin down streptavidin-peroxidase tubes before use. Dilute 120 µl of the desired conjugate to 12 ml with dilution buffer, which is enough for 1 x 96 tests. In case less test are required, prepare the required volume of conjugate solutions by diluting 1 part of the desired conjugate with 99 parts of dilution buffer.

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9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use. Working following sterile handling procedures is preferred.

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 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at 37°C.
5. Wash the plates 4 times with wash buffer using a plate washer or as follows*:
   a. Carefully remove the cover, 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. Add 100 µl of the appropriate diluted conjugate to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray and incubate the tray for 1 hour at 37°C
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 30 minutes at room temperature. It is advised to control the reaction on the plate regularly. In case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
11. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 9. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
12. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure.
Make sure the plate washer is used as specified for the manual method.

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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).
- 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. Samples should be referred to the standard curve prepared on the same plate.
- 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, peroxidise conjugate 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.
- 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 for inquiries and technical support regarding the EndoCab® ELISA.

Hycult Biotech, Frontstraat 2a, 5405 PB Uden, the Netherlands

T: +31 (0)413 251 335, F: +31 (0)413 248 353

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12. QUALITY CONTROL

The Certificate of Analysis 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 Analysis 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 sent to support@hycultbiotech.com.

Suggestions summarized below in Table 2 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
•					Lamp in microplate reader is not functioning optimally

Table 2

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14. REFERENCES

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