

LBP for a wide variety of species

HK503
Edition 08-16

**ELISA KIT
PRODUCT INFORMATION & MANUAL**

Read carefully prior to starting procedures!
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 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 LBP ELISA kit for a wide variety of species is to be used for the *in vitro* quantitative determination of LBP in cell culture supernatants, plasma or serum. Measurable species are amongst others: bovine, canine, human, monkey, rabbit, rat, sheep and swine. For quantification, human LBP is used as a reference standard. 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

Lipopolysaccharide (LPS) Binding Protein (LBP) is a type 1 acute phase protein that is constitutively produced by the liver and rapidly upregulated during the acute phase response. LBP plays a central role in the response to LPS. The protein catalyses the monomerization of LPS and its transfer to (s)CD14 and to lipoproteins. In this way LBP has both a role in the activation pathway of LPS: activation of monocytes by LPS leading to release of inflammatory mediators and in the neutralization of LPS i.e. the uptake of LPS by lipoprotein and subsequent clearing. The assay can be used to quantify functional LBP in tissue culture supernatants, plasma and serum samples of several species.

In plasma of healthy individuals LBP is present at levels of approximately 10 µg/ml, which increase approximately 10-fold during acute phase responses.

3. KIT FEATURES

- Working time of 3½ hours.
- Minimum concentration which can be measured is 1.6 ng/ml.
- Measurable concentration range of 1.6 tot 100 ng/ml.
- Working volume of 100 µl/well.

Cross-reactivity

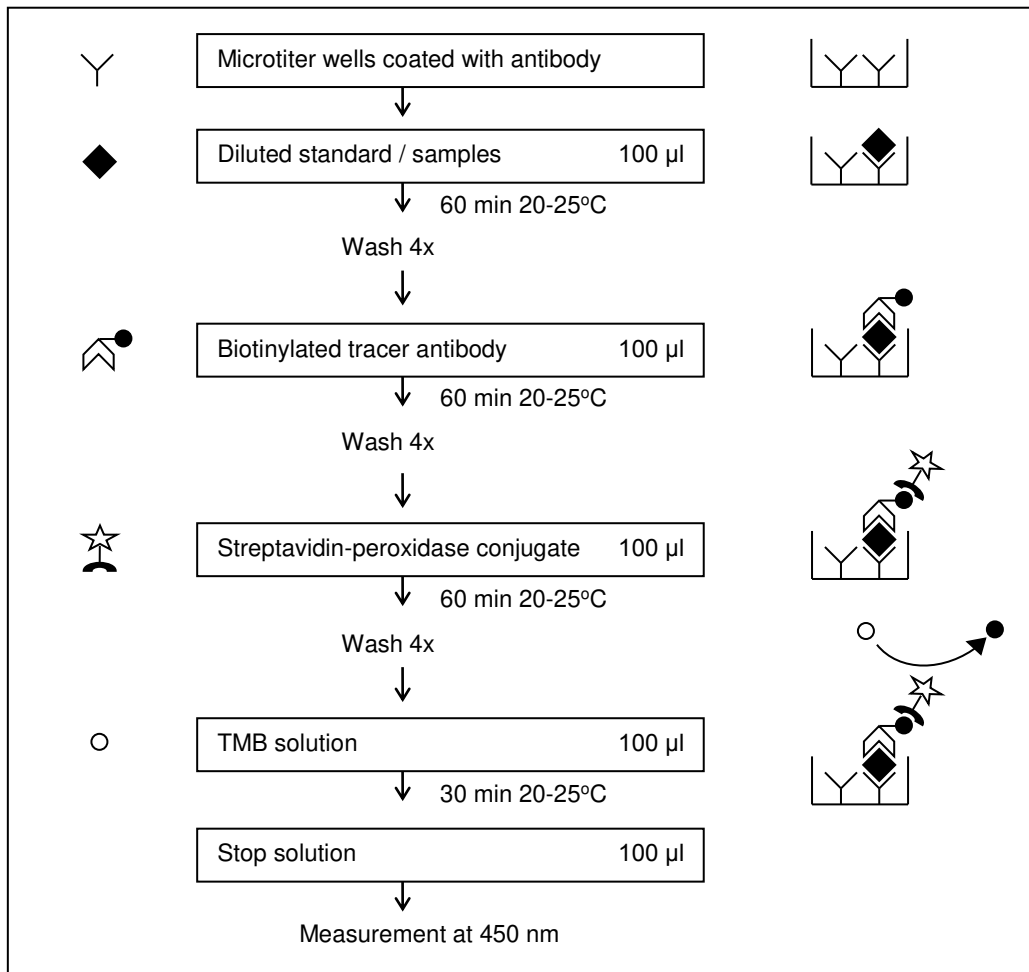
Potential cross-reacting proteins detected in the LBP for a wide variety of species ELISA:

Cross reactant	Reactivity
Bovine	Strong
Canine	Strong
Human	Strong
Monkey	Strong
Rabbit	Strong
Rat	Strong
Sheep	Strong
Swine	Strong

Table 1

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

4. PROTOCOL OVERVIEW



- The LBP ELISA kit for a wide variety of species is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ 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 antibodies recognizing LBP.
- Biotinylated tracer will bind to captured LBP.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer (LPS).
- Streptavidin-peroxidase conjugate 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 human LBP standards (log).
- The LBP concentration of samples, which are run concurrently with the standards, can be determined from the human LBP standard curve.

5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Cat.#	Quantity	Color code
Wash buffer 40x	WB01	1 vial (30 ml)	Colorless
Dilution buffer 10x	DB81	1 vial (15 ml)	Green
Standard		2 vials, lyophilized	White
Tracer, biotinylated 12x		2 vials, 1 ml lyophilized	White
Streptavidin-peroxidase 100x	CON03	1 vial, 0.25 ml in solution	Brown
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 2

- 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 and tracer in lyophilized form and the streptavidin-peroxidase in concentrated solution are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- The standard is single use. After reconstitution the standard cannot be stored.
- Once reconstituted, the biotinylated tracer is stable for 12 hours. Longer stability can not be guaranteed.
- The streptavidin-peroxidase can only be stored in concentrated solution and cannot be stored once 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.

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 add under any circumstances 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.
- 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 advise 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.
- 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. 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 (1,500xg 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 (1,500xg 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 LBP. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of LBP 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

LBP can be measured accurately if serum or plasma samples are diluted with supplied dilution buffer in polypropylene tubes according to Table 3:

Species	Advised dilution for plasma or serum
Bovine	≥ 1: 100
Canine	≥ 1: 10
Human	≥ 1: 100
Monkey	≥ 1: 100
Rabbit	≥ 1: 2
Rat	≥ 1: 10
Sheep	≥ 1: 10
Swine	≥ 1: 100

Table 3

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 LBP 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 LBP.

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.

Wash buffer

Prepare wash buffer by mixing 30 ml of wash buffer with 1170 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 wash buffer with 39 parts of distilled or de-ionized water.

Dilution buffer

Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 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 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 pipetting 0.25 ml of distilled or de-ionized water. After reconstitution add the amount of dilution buffer mentioned on the CoA to the vial. Use the standard vial as Tube 1 in Figure 1. Prepare each LBP standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*. After reconstitution the standard must be used within 1 hour. The standard can not be stored for repeated use.

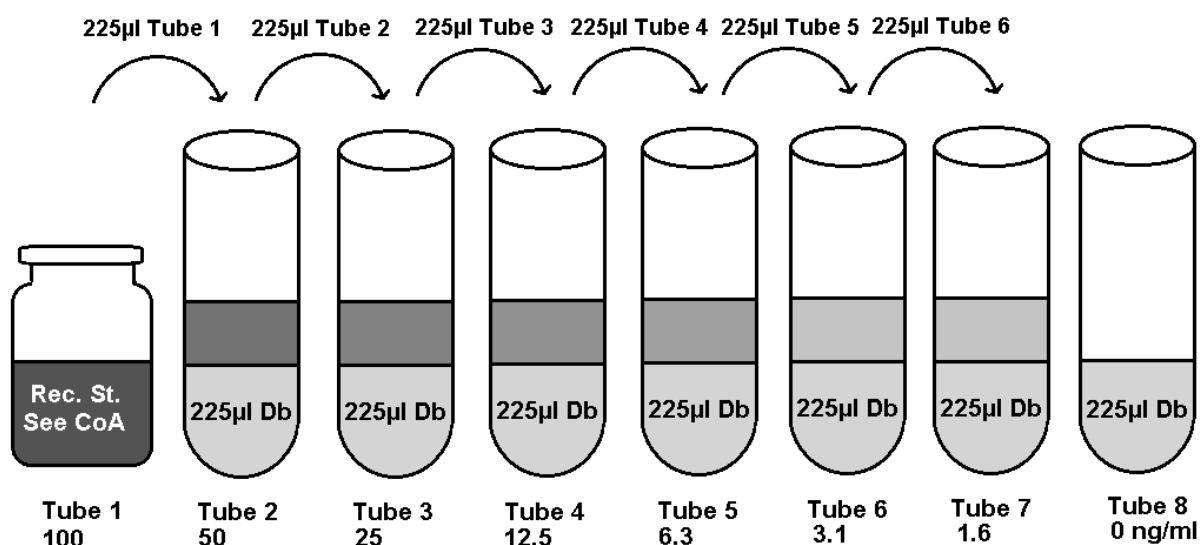


Figure 1

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

Biotinylated tracer

The biotinylated tracer (LPS) is reconstituted by pipetting 1 ml distilled or de-ionized water. Add 11 ml of the dilution buffer to a vial reconstituted biotinylated tracer, which is sufficient for 96 tests. In case less tests are required, prepare the required volume of biotinylated tracer: dilute 1 part of the reconstituted tracer with 11 parts of dilution buffer.

Streptavidin-peroxidase solution

Prepare the streptavidin-peroxidase solution by mixing 0.25 ml of the 100x streptavidin-peroxidase solution with 24.75 ml dilution buffer, which is sufficient for 2 x 96 tests. In case

less volume is required, prepare the desired volume of streptavidin-peroxidase solution by diluting 1 part of the 100x streptavidin-peroxidase solution with 99 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 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 room temperature.
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 diluted biotinylated tracer 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 room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase 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 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. 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.
13. Cover the tray and incubate the tray for 30 minutes at room temperature. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. 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.

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. Sample 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, biotinylated LPS, 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.
- 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 LBP (for a wide variety of species) ELISA.

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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.

The standard present in this assay is referred to the HK315 Human LBP ELISA.

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

13. PERFORMANCE CHARACTERISTICS

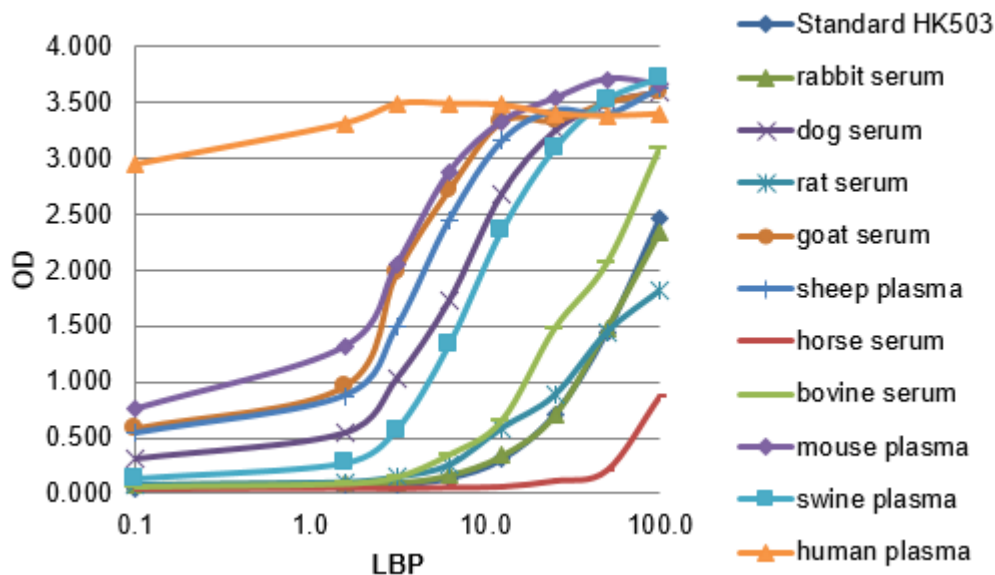
Inter and intra variation

The inter variation of the HK503 ELISA kit had an average CV of 7.9%. The lowest CV value was 2.3% and the highest 9.1%.

The intra variation had an average CV of 6.6%. The lowest CV value was 4.4% and the highest 8.4%.

Various species experiment

Samples of different species were tested in the HK503 ELISA kit. All tested species were suitable, however, optimal dilutions differ per sample type.



14. 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 shall be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 4 can be used as guideline in 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 adapted 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 4

15. REFERENCES

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