LBP
for a wide variety of species

HK503
Edition 02-24

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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Please note that the user protocol provided is not specific to any particular lot and represents the general specifications for this product. We advise consulting the vial label and the Certificate of Analysis for information regarding specific lots. Additionally, be informed that shipping conditions for this product may differ from its recommended storage conditions.

This product is intended solely for research purposes and is not approved for human or animal use, or for diagnostic procedures. Users must adhere to all applicable local, state, and federal regulations when utilizing this product. Hycult Biotech disclaims any liability for patent infringements that may arise from the use or adaptation 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 Binding Protein (LBP), a pivotal Type 1 acute phase protein primarily synthesized by the liver, plays a fundamental role in mediating the body's response to lipopolysaccharide (LPS), a key component of the outer membrane of Gram-negative bacteria. LBP is inherently involved in the mechanism of LPS response, facilitating the transformation of LPS into monomers and orchestrating its transfer to cell surface receptor (s)CD14 and various lipoproteins. This dual functionality positions LBP as a crucial player in both activating and neutralizing pathways of LPS response. It not only triggers the activation of monocytes, leading to the release of pro-inflammatory mediators but also contributes to the detoxification and clearance of LPS via lipoprotein-mediated uptake.

In healthy individuals, plasma LBP levels typically hover around 10 µg/ml, but these levels can surge by nearly tenfold during acute phase responses, reflecting its significant role in the body's defense mechanism. The LBP assay offers a robust method for quantifying functional LBP in an array of sample types, including tissue culture supernatants, plasma, and serum, across various species.

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
Mouse	Negative*

Table 1

* For the quantification of LBP in mouse species, the use of HK205: LBP, Mouse, ELISA kit (https://www.hycultbiotech.com/product/lbp-mouse-elisa-kit/) is highly recommended.

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

4. PROTOCOL OVERVIEW

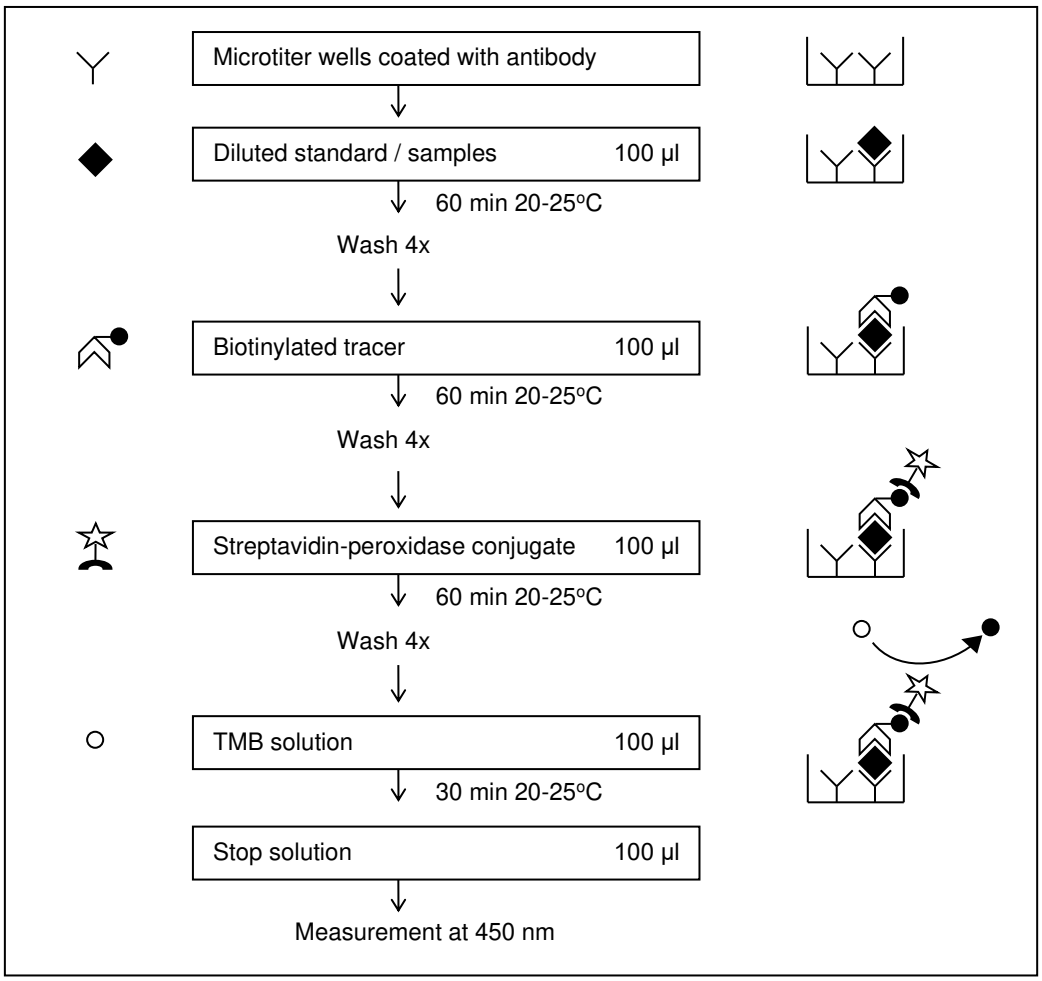

Figure 1

- 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

- Store the kit components at 2 - 8°C immediately upon receipt. Do not freeze.
- Ensure components are used before the expiration date indicated 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 quantity of the standard is specified on the vial label and the Certificate of Analysis.
- The standard is intended for a single use; it cannot be stored after reconstitution.
- After reconstitution, the biotinylated tracer remains stable for up to 12 hours. Stability beyond this duration is not assured.
- Store the streptavidin-peroxidase exclusively in its concentrated form; it is not stable once diluted.
- Ensure that the foil pouch containing the plate is vacuum-sealed and undamaged upon receipt. Any deviation may impact assay performance.
- Immediately return unused strips back into the foil pouch with the desiccant, sealing it fully along the entire edge of the zip-seal. Stored at 2 - 8°C, the quality is ensured for one month.

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

- This product is designated for research purposes only and is not intended for use in diagnostic or therapeutic procedures.
- Only qualified personnel trained in laboratory procedures should handle this kit.
- Under no circumstances should sodium azide be added to any component as a preservative.
- Refrain from using kit components beyond their expiration date.
- To ensure accuracy, do not interchange reagents from different kits or lots. Each kit and lot is calibrated as a complete unit; use only the reagents supplied by the manufacturer.
- The assay is specifically optimized for the stated standard range. Alterations to the standard range are not recommended.
- Exercise caution when opening vials as they are under vacuum.
- Avoid ingestion of any kit components.
- The kit reagents include 2-chloroacetamide, a preservative known for its harmful effects upon skin contact and toxicity if ingested. In the event of an accident or discomfort, immediate medical consultation is advised.
- Protect the TMB substrate from intense light exposure; it should remain colourless until utilized.
- The stop solution contains 2% oxalic acid, a substance that can irritate or burn the respiratory system, skin, and eyes. Avoid any direct contact, and in case of exposure, rinse thoroughly with water and seek medical attention.
- Deviations from the specified incubation times, temperatures, or pipetting volumes may result in inaccurate results.
- Once dispensed, avoid reusing microwells or returning reagents to their original bottles.
- Treat all biological samples as potentially hazardous or infectious and handle them under conditions that minimize the risk of disease transmission.
- Be aware that samples that are hemolyzed, hyperlipemic, heat-treated, or contaminated may yield inaccurate results.
- Utilize polypropylene tubes for the preparation of standards and samples, avoiding the use of polystyrene tubes or sample plates.
- The standard is derived from human sources and has been tested for various viruses with negative results. However, as no testing method can guarantee the complete absence of infectious agents, treat this reagent with the same precautions as you would any potentially infectious human serum or blood specimen. Follow established guidelines for preventing the transmission of blood-borne infections when handling materials in contact with this reagent.

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	Recommended 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:

- The recommended dilutions are not based on verified serum/blood values, but the dilutions demonstrate a recovery rate > 90%.
- 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 2. Prepare each LBP standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 2*. After reconstitution the standard must be used within 1 hour. The standard cannot be stored for repeated use.

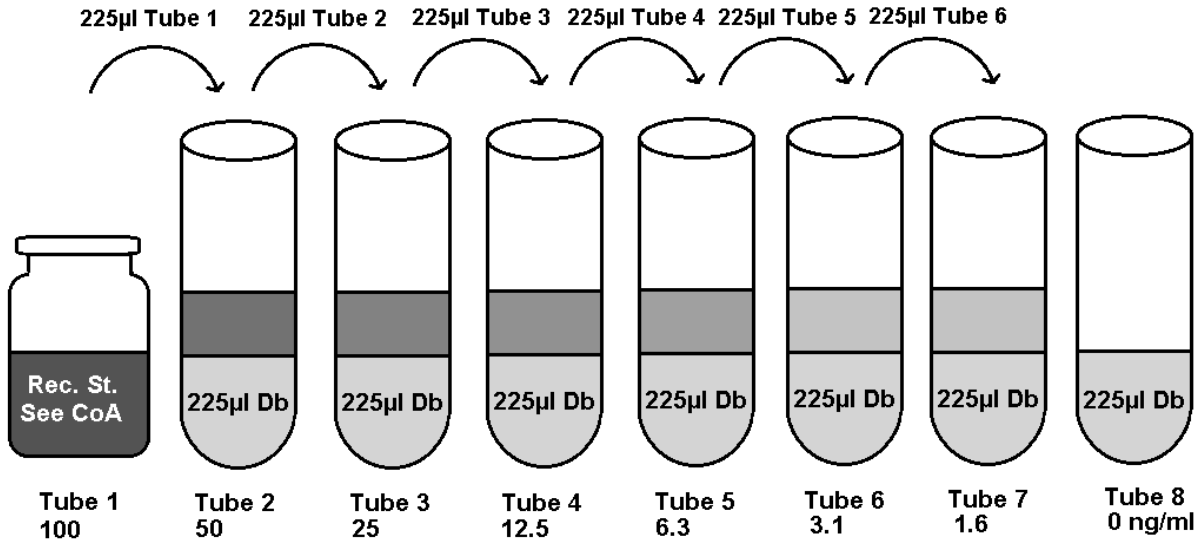

Figure 2

*) 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

- Determine the average absorbance for each group of duplicate standards, controls, and samples.
- Discrepancies exceeding 15% from the mean absorbance value suggest potential inaccuracies, necessitating sample reanalysis.
- Ensure the mean absorbance of the zero standard does not surpass 0.3.
- Utilize specialized software to construct a standard curve, plotting mean absorbance values (Y-axis) against corresponding concentrations (X-axis) on a logarithmic scale.
- For diluted samples, adjust the concentration derived from the standard curve by the dilution factor.
- Samples yielding an average absorbance higher than that of the maximum standard concentration exceed the assay's scope and must be reanalysed using a greater dilution factor.

11. TECHNICAL HINTS

- Technicians should be proficient and well-versed in ELISA assays and the specific test procedures before initiating the assay.
- For those unfamiliar with ELISA techniques, it is advisable to conduct a preliminary assay with a standard curve to ensure understanding and adherence to the protocol before proceeding with sample evaluations.
- Accurate and thorough washing is critical at all stages of the assay to prevent false positive or negative outcomes. Ensure complete removal of liquids from wells prior to adding wash buffer, adhere strictly to the specified volume for each washing cycle, and avoid allowing the wells to remain uncovered or dry for prolonged periods.
- A standard curve is essential for each assay run due to varying conditions; samples must be evaluated against a standard curve established on the same plate during that session.
- Do not interchange reagents from different kits or batches, including strips, and avoid combining remnants with contents from new vials.
- Prepare fresh dilutions of the standard, samples, biotinylated LPS, streptavidin-peroxidase, and buffers each time the kit is utilized.
- Maintain cap-to-vial correspondence; caps are designed to fit their original vials and should not be swapped.
- Prevent cross-contamination by using new pipette tips for each addition across standards, samples, and reagents, and employ separate reservoirs for each reagent to ensure integrity.
- Dispose of all waste in accordance with the established laboratory safety protocols and regulations.

Technical support

For any questions or technical support related to the LBP (for a wide variety of species) ELISA, please feel free to reach out to our technical support team at support@hycultbiotech.com.

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12. QUALITY CONTROL

The Certificate of Analysis accompanying this kit is specific to its lot number, intended to verify the results achieved in your facility. Please note that the absorbance values indicated on the Certificate serve merely as reference points; deviations in outcomes produced by your laboratory are to be expected. Designed to mitigate the impact of soluble receptors, binding proteins, and extraneous variables present in biological samples, this assay aims for precise measurement free from external interferences. Nonetheless, without exhaustive testing of all possible variables in the Hycult Biotech immunoassay framework, the exclusion of interference cannot be entirely assured.

The assay's standard is aligned with the HK315 Human LBP ELISA specification.

To ensure the highest efficacy of this kit, implementing good laboratory practices(GLP) is crucial.

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.

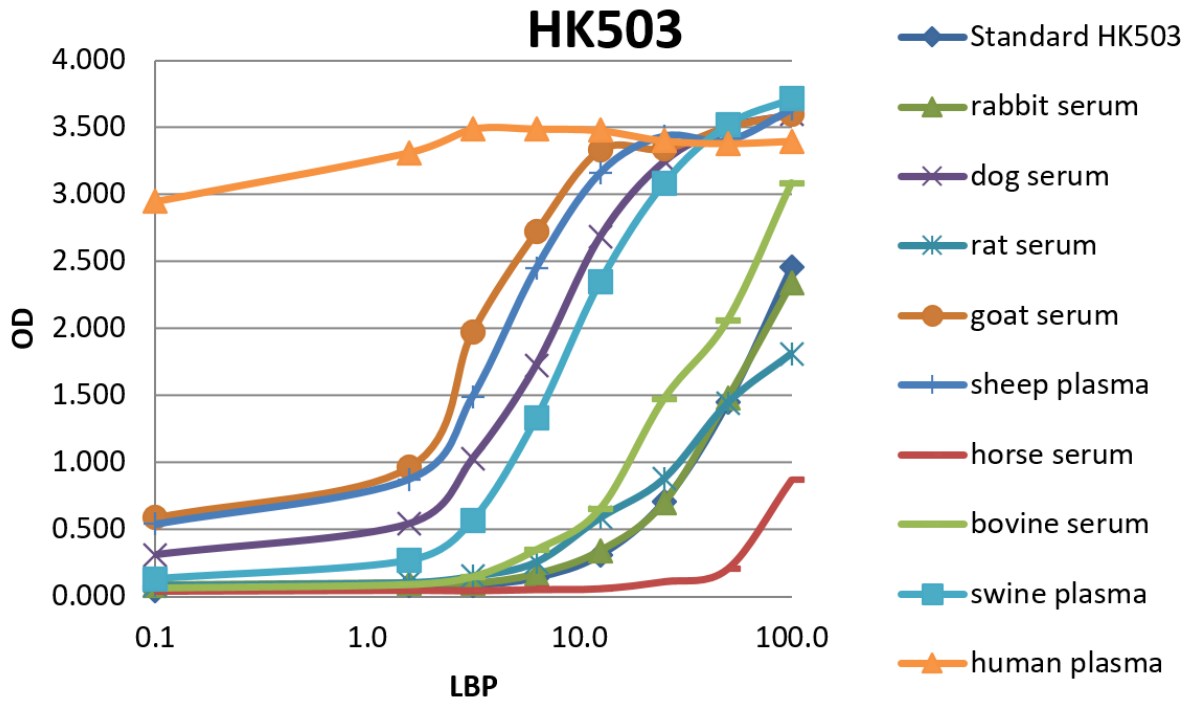

Figure 3

14. TROUBLESHOOTING

To ensure the highest level of customer satisfaction and to uphold the integrity of our products, we kindly request that any warranty claims or reports of deficiencies be submitted prior to the product's expiration date. Please include the lot number and relevant experimental data in your written communication, which should be directed to support@hycultbiotech.com for prompt assistance.

The recommendations provided in Table 4 serve as a structured guide for addressing unforeseen outcomes in 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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