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ab279407
Human LBP SimpleStep ELISA® Kit (LPS Binding Protein)

For the quantitative measurement of human LBP in serum, plasma, and cell culture supernatant samples.

This product is for research use only and is not intended for diagnostic use.

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Table of Contents

1. Overview 3
2. Protocol Summary 4
3. Precautions 5
4. Storage and Stability 5
5. Limitations 6
6. Materials Supplied 6
7. Materials Required, Not Supplied 7
8. Technical Hints 8
9. Reagent Preparation 9
10. Standard Preparation 10
11. Sample Preparation 11
12. Plate Preparation 12
13. Assay Procedure 13
14. Calculations 15
15. Typical Data 16
16. Typical Sample Values 17
17. Assay Specificity 21
18. Species Reactivity 22
19. Troubleshooting 23

Technical Support 24

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1. Overview

LBP *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of LBP protein in human serum, plasma, and cell culture supernatant samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

LBP (LPS Binding Protein) is an acute phase protein involved in the cellular response to gram-negative bacterial infections. LBP binds to LPS protein expressed on gram-negative bacteria and elicits a response by engaging with pattern recognition receptors, CD14 and TLR4. LBP is essential for the initial response to gram-negative bacterial infection but not for the downstream stages.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 50 µL standard or sample to appropriate wells



Add 50 µL Antibody Cocktail to all wells



Incubate at room temperature for 1 hour



Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT



Add 100 µL TMB Development Solution to each well and incubate for 10min.



Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity 1x96 tests	Quantity 10x96 tests	Storage Condition
Human LBP Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human LBP Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human LBP Lyophilized Recombinant Protein	2 Vials	10 x 2 vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. Standard Preparation

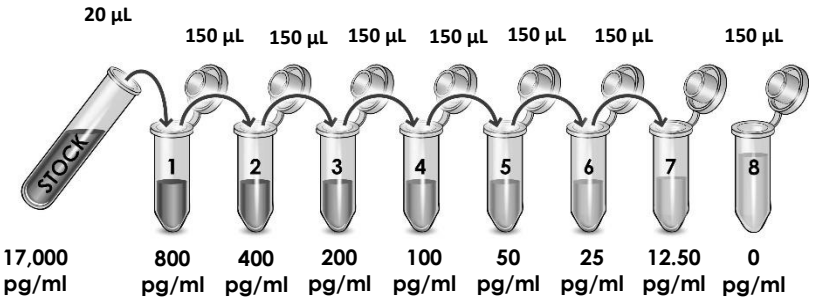
- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the LBP by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the LBP standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 17,000 pg/ml **Stock Standard** Solution.

10.1.1 Label eight tubes, Standards 1– 8.

10.1.2 Add 405 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.1.3 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1: 64,000 - 1: 4,000
Plasma - Citrate	1: 64,000 - 1: 2,000
Plasma - Heparin	1: 64,000 - 1: 2,000
Plasma - EDTA	1: 64,000 - 1: 2,000
1640 RPMI Cell Culture Media*	≤ 50%

*Based on spiked sample

11.1 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1: 4,000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1: 2,000 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture supernatant samples at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute samples at least 1: 2 into Sample Diluent NS, and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 60 min at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10min in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

- **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

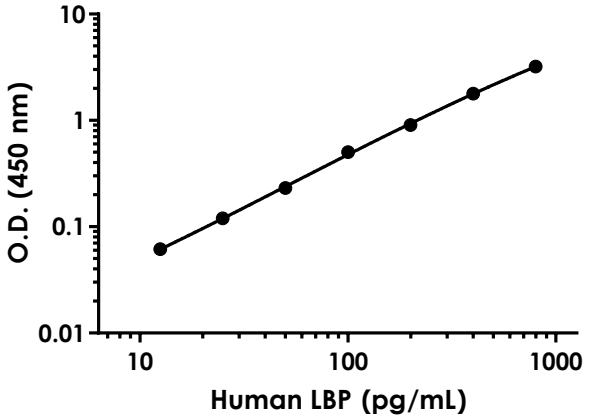
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4- parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (pg/ml)	O.D 450 nm		Mean O.D
	1	2	
0	0.055	0.059	0.057
12.50	0.123	0.116	0.119
25	0.184	0.172	0.178
50	0.289	0.289	0.289
100	0.602	0.522	0.562
200	0.948	0.972	0.960
400	1.835	1.845	1.840
800	3.280	3.259	3.270

Figure 1. Example of human LBP standard curve in Sample Diluent NS. The LBP standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 6.79 pg/ml. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of LBP Recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Serum (1: 32,000)	106	97 - 112
Plasma – Citrate (1: 32,000)	94	88 - 100
Plasma – Heparin (1: 32,000)	100	93 - 108
Plasma – EDTA (1: 32,000)	95	93 - 97
1640 RPMI (50%)*	90	86 - 93

*Media is RPMI 1640 without fetal bovine serum. Media containing fetal bovine serum is not recommended with this kit.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native LBP was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in NS.

Dilution Factor	Interpolated value	1: 4,000 Serum	1: 2,000 Plasma (Citrate)	1: 2,000 Plasma (Heparin)	1: 2,000 Plasma (EDTA)
Undiluted	**pg/ml**	556	773	527	741
	% Expected value	100	100	100	100
2	**pg/ml**	252	365	241	348
	% Expected value	91	94	91	94
4	**pg/ml**	123	173	126	175
	% Expected value	89	89	95	94
8	**pg/ml**	60	85	63	91
	% Expected value	87	88	96	98
16	**pg/ml**	29	41	31	44
	% Expected value	84	85	94	94

Recombinant LBP was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% 1640 RPMI Cell Culture Media
Undiluted	**pg/ml**	385
	% Expected value	100
2	**pg/ml**	187
	% Expected value	97
4	**pg/ml**	96
	% Expected value	100
8	**pg/ml**	48
	% Expected value	99
16	**pg/ml**	22
	% Expected value	90

PRECISION –

Mean coefficient of variations of interpolated values of LBP from two concentrations of serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	4.4	6.0

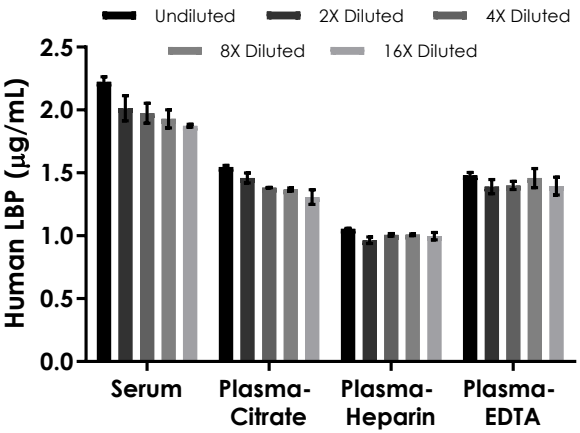


Figure 2. Interpolated concentrations of native LBP in human serum and plasma samples. The concentrations of LBP were measured in duplicates, interpolated from the LBP standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1: 4,000, plasma (citrate) 1: 2,000, plasma (heparin) 1: 2,000, and plasma (EDTA) 1: 2,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean LBP concentration was determined to be 2.00 µg/mL in serum, 1.39 µg/mL in plasma (citrate), 1.01 µg/mL in plasma (heparin), and 1.50 µg/ml in plasma (EDTA).

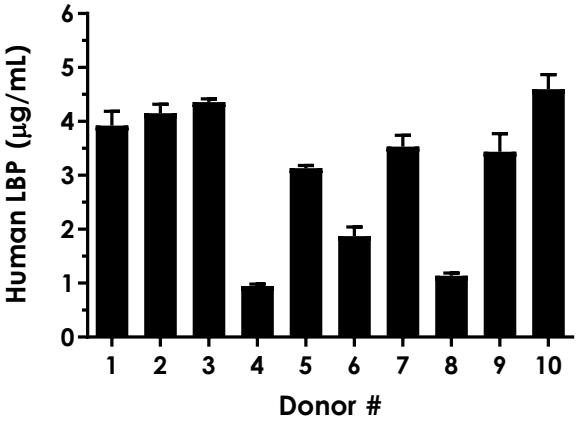


Figure 3. Serum from ten individual healthy human female donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean LBP concentration was determined to be 3.11 µg/mL with a range of 0.94 – 4.60 µg/ml.

17. Assay Specificity

This kit recognizes both native and recombinant human LBP protein in serum, plasma, and cell culture media samples only.

Cell and tissue extracts, urine, milk, saliva, and cerebrospinal fluid samples have not been tested with this kit.

This kit is incompatible with cell culture samples containing fetal bovine serum unless the bovine serum is sufficiently diluted. User will need to determine what level of dilution is required for their bovine serum to avoid signal. Serum-free media is recommended.

CROSS REACTIVITY

Recombinant human CD14 was prepared at 50 ng/mL and 5 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human CD14 was prepared at 50 ng/mL and 5 ng/mL and tested for interference. No interference with recombinant standard protein was observed.

Lipopolysaccharides (LPS) were prepared at 300 ng/mL and 4.70 ng/ml and tested for interference. 300 ng/ml LPS reduced the interpolated value of 200 pg/mL of recombinant LBP standard protein 11.45-fold (91%). 4.70 ng/mL LPS reduced the interpolated value of 200 pg/mL of recombinant LBP standard protein 1.34-fold (25%).

18. Species Reactivity

This kit recognizes human LBP protein.

Other species reactivity was determined by measuring 1: 16,000 mouse, rat, and bovine serum samples, interpolating the LBP protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the LBP protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Other species reactivity not determined.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Technical Support

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