Lipopolysaccharides ELISA Kit

User Manual

Catalog # CEK3053

(Version 1.1A)

Competitive Inhibition Enzyme Immunoassay for quantitative detection of Lipopolysaccharides concentrations in Serum, Plasma, Tissue Homogenates, Cell Lysates, Cell Culture Supernates or Other Biological Fluids.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lipopolysaccharides (LPS) are important outer membrane components of gram-negative bacteria. They are large amphipathic glycoconjugates that typically consist of a lipid domain (hydrophobic) attached to a core oligosaccharide and a distal polysaccharide. These molecules are also known as lipogylcans due to the presence of lipid and sugar molecules.

II. ASSAY PRINCIPLES

Cohesion Biosciences Lipopolysaccharides ELISA Kit (Competitive Inhibition Enzyme Immunoassay) is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of Lipopolysaccharides in Serum, Plasma, Tissue Homogenates, Cell Lysates, Cell Culture Supernates or Other Biological Fluids. This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to Lipopolysaccharides has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled Lipopolysaccharides and unlabeled Lipopolysaccharides (standards or samples) with the pre-coated antibody specific to Lipopolysaccharides. After incubation the unbound conjugate is washed off. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of Lipopolysaccharides in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of Lipopolysaccharides in the sample.

III. KIT COMPONENTS

Component	Volume
96-well Plate Pre-coated with Anti-Lipopolysaccharides Antibody	8 wells x 12 Strips
Lipopolysaccharides Standard	200 ng x 2
Biotin-Labeled Competitive Inhibitor (100X)	60 µl
Streptavidin-HRP (100X)	120 µl
Standard/Sample Diluent	30 ml
Biotin-Labeled Competitive Inhibitor Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
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IV. STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.

V. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and pipette tips to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

VI. HEALTH AND SAFETY PRECAUTIONS

1. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
2. Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

VII. REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 X g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

Cell Lysates: Collect cells and rinse cells with PBS. Homogenize and lyse cells throughly in lysate solution. Centrifuge cell lysates at approximately 10000 X g for 5 minutes to remove debris. Aliquots of the cell lysates were removed and assayed.

Bone Tissue: Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M Guanidine-HCl.

Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed. Alternatively, aliquot and store samples at ≤ -20 °C.

Note: Some lysis buffer, such as RIPA can not be used. Some components will affect the binding.

Urine: Urinary samples should be cleared by centrifugation and then can be used directly without dilution. Storage at -20°C.

2. Standard Preparation

Reconstitute the lyophilized Lipopolysaccharides Standard by adding 1 ml of Standard/Sample Diluent to make the 200 ng/ml standard stock solution. Allow solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Two tubes of the standard (200 ng per tube) are included in each kit. Use one tube for each experiment.

Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (3.12 ng/ml - 200 ng/ml) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 ng/ml).

Standard	Add	Into
200 ng/ml		
100 ng/ml	500 µl of the Standard (200 ng/ml)	500 µl of the Standard/Sample Diluent
50 ng/ml	500 µl of the Standard (100 ng/ml)	500 µl of the Standard/Sample Diluent
25 ng/ml	500 µl of the Standard (50 ng/ml)	500 µl of the Standard/Sample Diluent
12.5 ng/ml	500 µl of the Standard (25 ng/ml)	500 µl of the Standard/Sample Diluent
6.25 ng/ml	500 µl of the Standard (12.5 ng/ml)	500 µl of the Standard/Sample Diluent
3.12 ng/ml	500 µl of the Standard (6.25 ng/ml)	500 µl of the Standard/Sample Diluent
0 ng/ml	1 ml of the Standard/Sample Diluent	

Note: The standard solutions are best used within 2 hours. The 200 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Biotin-Labeled Competitive Inhibitor Working Solution Preparation

The Biotin-Labeled Competitive Inhibitor should be diluted in 1:100 with the Biotin-Labeled Competitive Inhibitor Diluent and mixed thoroughly. The solution should be prepared no more than 2 hours prior to the experiment.

4. Streptavidin-HRP Working Solution Preparation

The Streptavidin-HRP should be diluted in 1:100 with the Streptavidin-HRP Diluent and mixed thoroughly. The solution should be prepared no more than 1 hour prior to the experiment.

5. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 600 ml with glass-distilled or deionized water (1:20).

VIII. ASSAY PROCEDURE

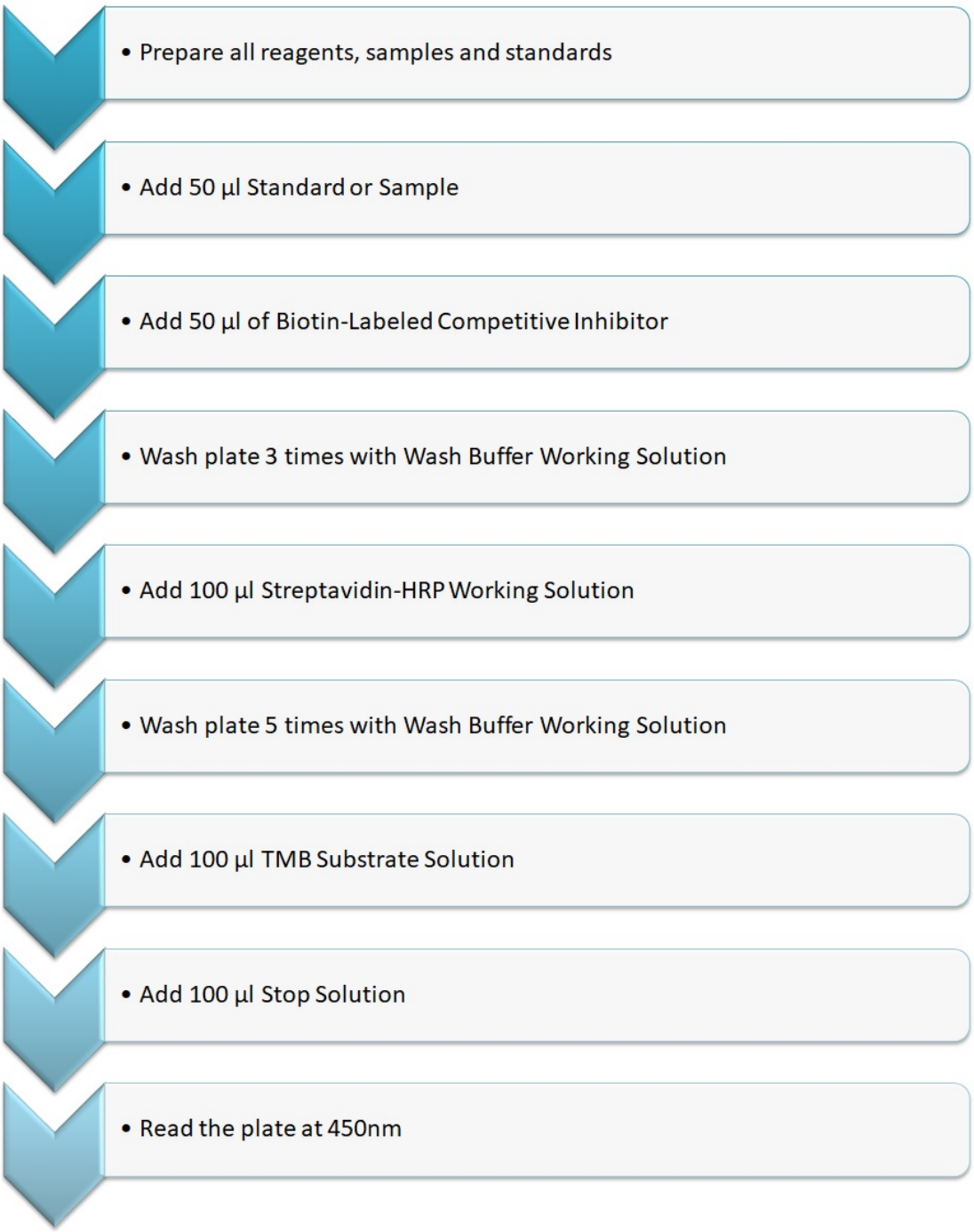
The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of protein amount in samples.

1. Prepare 7 wells for standard, 1 well for blank. Add 50 µl of each standard and samples into appropriate wells. Then add 50 µl of Biotin-Labeled Competitive Inhibitor Working Solution to each well immediately.
2. Cover well and incubate for 60 minutes at room temperature with gentle shaking (using a microplate shaker is recommended).
3. Remove the cover, discard the solution and wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 100 µl of Streptavidin-HRP Working Solution into each well and incubate the plate at 37°C for 45 minutes.
5. Wash plate 5 times with Wash Buffer Working Solution, and each time let wash buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.
6. Add 100 µl of TMB Substrate Solution into each well and incubate plate at 37°C in dark for 10 - 20 minutes.
7. Add 100 µl of Stop Solution into each well. The color changes into yellow immediately.
8. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

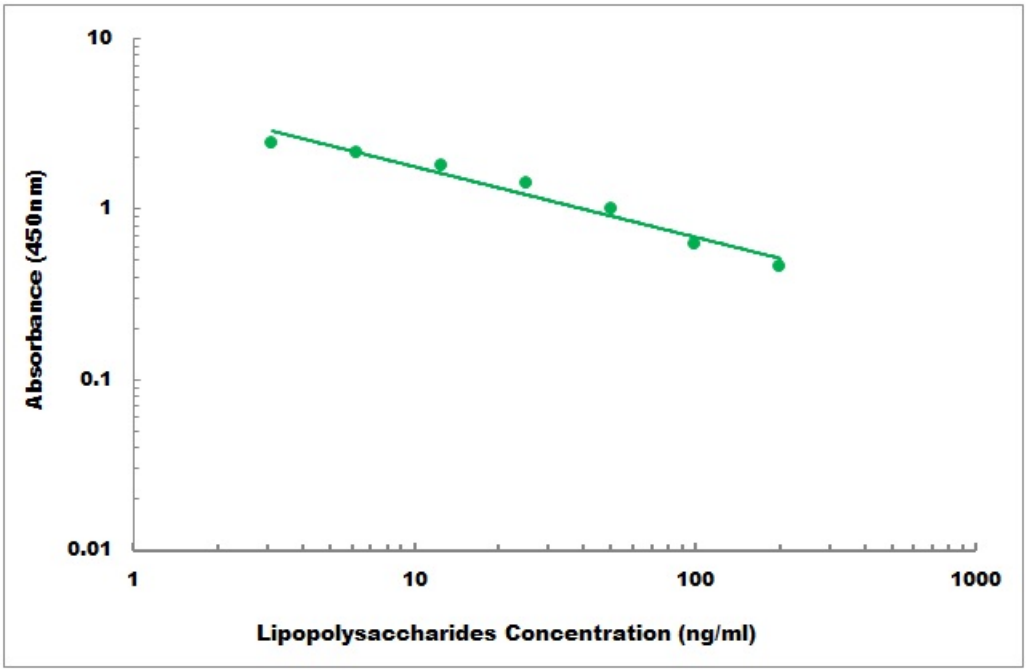
Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

IX. ASSAY PROCEDURE SUMMARY



X. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



XI. SENSITIVITY

The minimum detectable dose of Lipopolysaccharides is typically less than 1.24 ng/ml.

XII. SPECIFICITY

Lipopolysaccharides ELISA Kit has high sensitivity and excellent specificity for detection of Lipopolysaccharides.

The detection range is 3.12 ng/ml - 200 ng/ml.

XIII. CROSS REACTIVITY

No significant cross-reactivity or interference between Lipopolysaccharides and analogues was observed.

XIV. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much Streptavidin-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20 - 30 °C before performing assay
Too much signal-whole plate turned uniformly blue	• Insufficient washing-unbound Streptavidin-HRP remaining	• Increase number of washes Carefully
	• Too much Streptavidin-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual Streptavidin-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample Recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run Again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

XV. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

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XVI. NOTES