Package Insert EndoLISA®

Cat. No. 609033
192 tests

For laboratory and research use only. Not for use in diagnostic procedures.

Store the kit at 2 to 8°C

Table of Contents

Abbreviations used:

DMSO	Dimethyl sulfoxide
EDTA	Ethylene diamine tetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
EU	Endotoxin Unit (1 EU corresponds to 0.1 ng LPS (FDA RSE *E. coli* O113 EC-6)
LAL	Limulus amebocyte lysate
LLOQ	Lower limit of quantification
LPS	Lipopolysaccharide
MTP	Microtiter plate
rfu	Relative fluorescence unit
rpm	Revolutions per minute
RSE	Reference Standard Endotoxin

1. General Information

1.1 Intended Use

Intended use

EndoLISA® is intended for *in vitro* quantitative determination of lipopolysaccharide (LPS) in pharmaceutical and biological end-products, in-process control and research samples.

Warning: For *laboratory and research* use only. EndoLISA® is not intended for the diagnosis of human or animal diseases.

Note: EndoLISA® is not suitable for the detection of endotoxin in serum, plasma and blood samples.

Number of tests

The kit contains reagents for 192 tests.

1.2 Kit Components

Kit components

Component	Container	Content	Description
1 EndoLISA® Plate	Aluminium bag	2 plates	Pre-coated microplates; a total of 12 modules with 16 wells each
2 Water (endotoxin-free)	Plastic bottle, blue cap	2 x 60 ml	Endotoxin-free water for reconstitution of the standard and dilution of samples and standard
3 Binding Buffer (6x)	Glass bottle, white cap	2 x 2.5 ml	Binding buffer, 6-fold concentrated, ready-to-use
4 Endotoxin Standard	Glass bottle, red cap	2 bottles	Endotoxin standard, lyophilized, containing approx. 1000 EU of LPS from *E. coli* O55,:B5
5 Wash Buffer	Plastic bottle, yellow cap	2 x 75 ml	Wash buffer; ready-to-use
6 Enzyme	Plastic bottle, transparent cap	2.5 ml	Enzyme solution for the detection of LPS, 10-fold concentrated
7 Substrate	Brown plastic bottle, brown cap	2.5 ml	Fluorescence substrate, 10-fold concentrated
8 Assay Buffer	Brown plastic bottle, brown cap	2 x 12 ml	Assay Buffer, to be combined with Substrate (7) and Enzyme (6)
9 Cover Foil		2 pieces	Adhesive cover foils to be used during binding step

1.3 Specifications

Assay range

0 to 500 EU/ml

Sensitivity

LLOQ = 0.05 EU/ml

Assay time

3 hours and 30 minutes

Storage and stability

Unopened kits are stable at 2 to 8°C until the expiry date printed on the label. For more information on storage and stability of the individual components, please refer to the table in chapter 5.

2. Safety Information

Safety precautions

The kit reagents are not considered to be hazardous. The toxicological properties of the reagents provided with the kit have not been tested.
A Material Safety Data Sheet according to EC Directive 91/155/EEC is available at www.endolisa.com

3. Avoiding Contaminations

Endotoxin-free conditions

All materials used, such as containers and pipette tips, should be purchased endotoxin-free.

Important: For preparing samples and standard dilutions, glass test tubes should be used, since endotoxin may adhere to hydrophobic plastic surfaces.

Treatment of glass materials

After standard cleaning procedure, glass materials should be rinsed with ultrapure water and have to be "baked" at 200°C for 4h. Use aluminum caps or aluminum foil to cover openings.

Handling of sample material

Samples should be stored refrigerated or frozen. Treat samples carefully in order to avoid microbial or endotoxin contamination. All materials in direct contact with the specimen or test reagents must be endotoxin-free.

4. Instrumentation and Software

Vortex

To reconstitue the LPS standard mix thoroughly by vortexing at 1400 rpm for 10 minutes. Sample dilutions and standard dilutions should be mixed vigorously for 2 minutes. This is best achieved by using a Multi-tube Vortex (e.g. Heidolph Multi Reax test tube shaker).

Incubator / plate shaker

The incubation steps of EndoLISA® are performed at 37°C. Thorough mixing substantially improves binding kinetics and reduces the standard deviation of replicates.

Fluorescence reader

Microplate fluorescence readers from different suppliers may be used for reading of EndoLISA® results. EndoLISA® has been developed and validated on an FLx800™ reader supplied by BioTek Instruments (www.biotek.com). All validation results have been generated on this instrument with settings as described below. Using other hardware, these instrument parameters may be used as orientation but should be validated.

Instrument settings:	Temperature	37°C
	Excitation filter (nm/band)	380/20
	Emission filter (nm/band)	440/40
	Reading orientation	from top
	Readings per well	10
	Shaking mode	off
	Sensitivity (scale = 0% to100 %)	5-10% at 5 EU/ml

Adjustment of instrument sensitivity (gain)

Fluorescence detection provides a dynamic range of 4 orders of magnitude. When performing EndoLISA® for the first time, the sensitivity setting (gain) of the reader has to be adjusted. The optimal slope for the standard curve is achieved when the signal of the third standard (5 EU/ml) is adjusted between 5% and 10% of the maximum detectable signal of the reader. For example, the range of the FLx800™ Reader is between 0 to 99.000 rfu. For this instrument, the third standard should be adjusted between 5.000 rfu and 10.000 rfu.

Calculation software

For standard curve fitting and calculation of the endotoxin content of unknown samples a calculation software is required. Most preferably, the EndoLISA® data of the standard curve should be modelled by a 4 parameter logistic function. Alternatively, a linear model can be used.

When using the FLx800™ reader from BioTek Instruments, the **Gen5™ software** for standard curve calculation should be used. Using this software the software settings can be downloaded on the EndoLISA® website: www.endolisa.com
(Attention: The gain settings must be adjusted for each reader individually)

5. Reagent Preparation

Use of kit components, stability and storage conditions

Reagent	Preparation	Stability and storage conditions of working solutions
1: EndoLISA® Plate	Open sealed bag and remove the frame with the desired number of strips. Store unused strips in the reclosable zipper bag.	Once opened the strips are stable for 3 months when stored dry at 2-8°C
2: Water (endotoxin-free)	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
3: Binding Buffer 6x	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
4: Endotoxin Standard (*E. coli* O55:B5)	The reconstitution volume is printed on the label; resolve lyophilized standard with water (bottle 2); mix for at least 10 minutes while vortexing vigorously.	Stable for 1 week when stored at 2-8°C or until expiry date of the kit when stored frozen in aliquots at -20°C. Freeze and thaw only once.
5: Wash Buffer	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
6: Enzyme	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
7: Substrate	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
8: Assay Buffer	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
9: Cover Foils	Self-adhesive foils for single use; cut off the required size and remove protective membrane	Stable

Reagents to be prepared from kit components

Reconstitution of LPS Standard:

- The volume to be used for reconstitution of the LPS Standard (bottle 4) is indicated on the label.
- For reconstitution, pipette the indicated amount of endotoxin-free Water (bottle 2) into bottle 4.

  Important: Use new pipette tips for every pipetting step to avoid contamination of the endotoxin-free water.

- Close the bottle, mix thoroughly by vortexing at 1400 rpm for 10 minutes.

Assay Reagent:

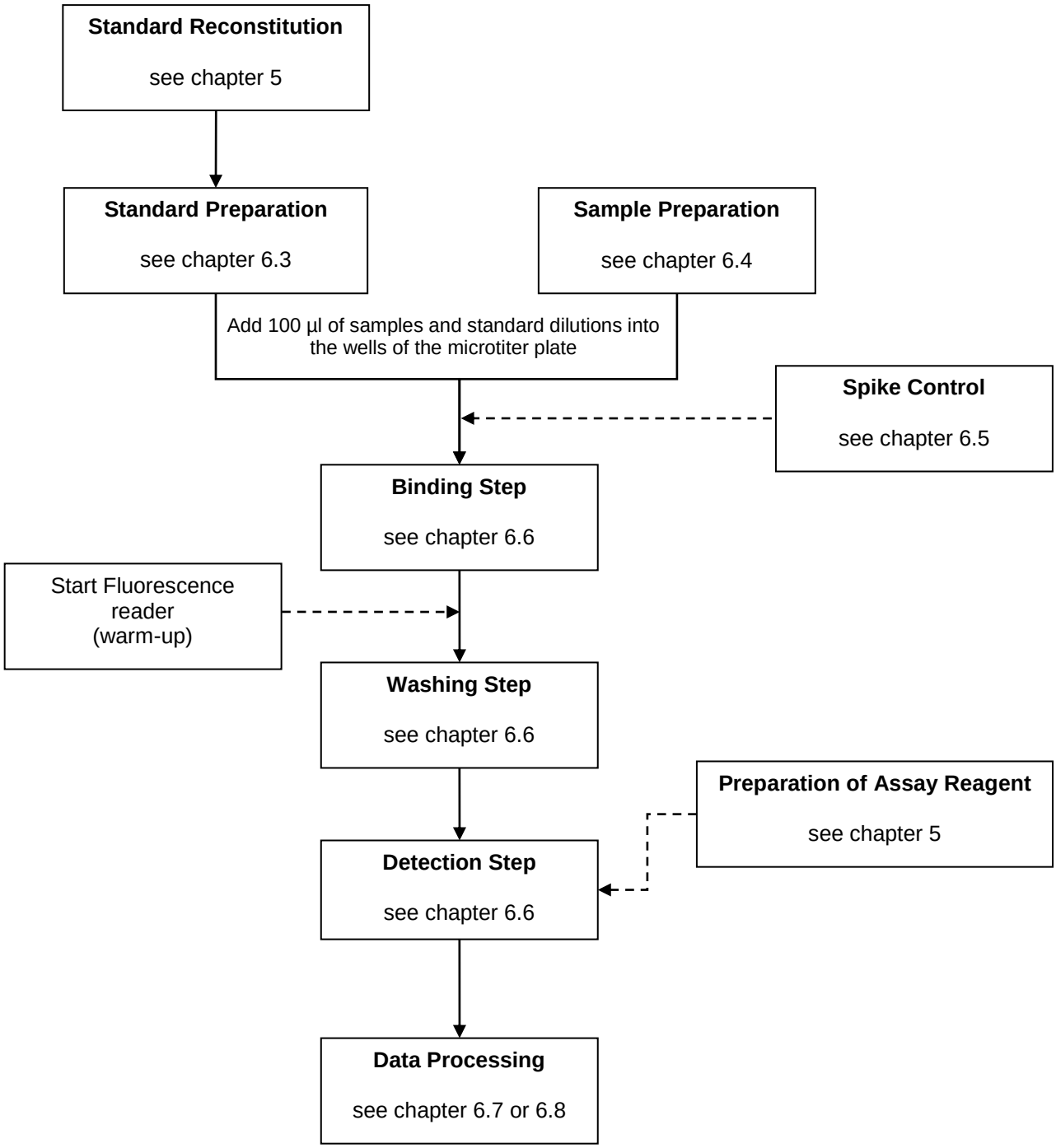
The Assay Reagent should be prepared shortly before use in the detection step (see chapter 6.6 Assay Procedure).
Required amounts are indicated in the table below. Combine 8 parts of Assay Buffer (bottle 8), 1 part of Substrate (bottle 7) and 1 part of Enzyme (bottle 6). Mix carefully - do not vortex.

Assemble the indicated volumes in an endotoxin-free reagent reservoir:

Assay Reagent	Assay Buffer	Substrate	Enzyme
2 ml for 16 reactions	1.6 ml	0.2 ml	0.2 ml
4 ml for 32 reactions	3.2 ml	0.4 ml	0.4 ml
6 ml for 48 reactions	4.8 ml	0.6 ml	0.6 ml
8 ml for 64 reactions	6.4 ml	0.8 ml	0.8 ml
10 ml for 80 reactions	8.0 ml	1.0 ml	1.0 ml
12 ml for 96 reactions	9.6 ml	1.2 ml	1.2 ml

6. Assay Protocol

6.1 Overview Assay Procedure



6.2 General Handling Instructions

Handling instructions

- All reagents needed for running EndoLISA® are supplied with the kit.
- Be careful not to contaminate the kit components in use.
- Let all reagents reach room temperature (20-25°C) before use.
- Pipette carefully to ensure accurate transfer of the small volumes.
- Perform a standard curve in parallel to each test series.
- Perform all measurements in duplicates.
- Reagents and MTP modules from different lots **MUST NOT** be mixed and used in one test series.

Equipment required

- Pipettes
- Multi channel pipette
- Dispensing pipette
- Pipette tips, endotoxin-free
- Dispensing pipette tips, endotoxin free
- Glass test tubes, endotoxin-free
- Reagent reservoir, endotoxin-free

Instruments

- Vortexer, 0-1500 rpm
- Incubator 37°C
- Plate shaker, 0-800 rpm
- Fluorescence reader, temperature controlled

6.3 Standard Preparation

Serial dilution of LPS Standard:

- The reconstituted LPS Standard has a concentration of 500 EU/ml (= first standard concentration). For reconstitution of the Endotoxin Standard see section 5.
- For preparation of the dilution series use endotoxin-free glass test tubes.

Important: Dilution in plastic vials may lead to poor recovery at lower concentrations.

- Pipette 900 µl of endotoxin-free water into each tube prepared for the dilution series.
- Add 100 µl of the reconstituted LPS Standard to prepare the second standard. Close the vial and mix thoroughly by vortex at 1400 rpm for 2 minutes (resulting concentration is 50 EU/ml).
- Repeat the subsequent 1:10 dilution steps accordingly to prepare the remaining concentrations.

Important: Vortexing at 1400 rpm between each dilution step as well as before pipetting into the MTP is essential to generate reliable results.

- Use endotoxin free water as zero standard (blank).
- Standard dilutions are stable for 8h when stored at 2-8°C.

Standard concentrations : Depending on the used calculation method different standard concentrations have to be prepared:

	Non-linear regression model	Linear regression model
500 EU/ml	+	-
50 EU/ml	+	+
5 EU/ml	+	+
0.5 EU/ml	+	+
0.05 EU/ml	+	+
0.005 EU/ml	optional	-
0 EU/ml	-	+

6.4 Sample Preparation

Sample preparation/ sample dilution

Many samples can be analyzed undiluted, but certain substances, concentrated samples and complex samples may disturb the assay (see section 6.10). In these cases a sample dilution of 1:5 in endotoxin-free Water (2) is recommended.

- For sample dilution use endotoxin-free glass test tubes.
- Pipette 400 µl of enodtoxin-free Water (2) into a vial and add 100 µl of sample. Vortex for 2 minutes.

Important: Protein concentration in samples should not exceed 5 mg/ml.

6.5 Spike Control

Spiking of samples

Spiking of samples can be used to validate if sample components interfere with the assay and dilution is required (see section 6.10 for interference parameter).

Spike material

The LPS Standard provided with the kit can be used for spike control.

Spike concentration

The LPS concentration of the spike should ideally be in the range of the expected LPS content of a given sample. It is recommended to use a spike concentration of 5 EU/ml for the samples.

Example:

- Add 10 µl of the 50 EU/ml standard to a sample (spike = 5 EU/ml).

Validity criteria

A result can be considered as valid, if the spike recovery is in the range of 50% to 200%. Samples with insufficient spike recovery have to be diluted.

Recommended protocol

- Pipette four replicates of the sample, 100 µl each.
- Add 10 µl of the 50 EU/ml standard to two of the four wells.
- Mix plate on a plate shaker for 2 minutes at 800 rpm.
- Proceed as described in section 6.6 binding step

Important: Make sure that the standard used for spiking is vortexted prior to use.

6.6 Assay Procedure

Filling of MTP

- Select the required number of strips and fix it into the frame. Duplicate determinations are recommended.
- Pipette 100 µl of sample or standard-dilution into the respective wells. The samples and the standard-dilution must be mixed thoroughly shortly before pipetting into the respective wells.
- If required perform spiking as described in section 6.5

Important: Make sure that sample and standard are vortexted prior to use.

Binding step

- Add 20 µl of 6x Binding Buffer to each well of standard and sample. Pipette carefully and use fresh tips to avoid cross-contamination.
- Seal the wells with cover foil.
- Incubate plate at 37°C for 90 minutes with continuous mixing at 450 rpm.

Important: Complex or concentrated samples may require a prolonged binding step (90 min to 18h) since LPS tends to interact with protein or amphiphilic components of the matrix and therefore requires more time to reach binding equilibrium

Start reader

- Be sure to start the fluorescence reader in time in order to reach the working temperature of 37°C.

Washing step

- Pour out the liquid by rapidly inverting the plate and dashing the liquid into a basin.
- Carefully pat dry the plate on a paper towel (top down). **Do not knock the plate** on the bench in any case.
- Add 150 µl of Wash Buffer (5) to each well using a multi-channel pipette.
- Repeat the washing step twice.
- Finally pour out the liquid by rapidly inverting the plate and dashing the liquid into a basin.
- Carefully pat the plate dry on a paper towel (top down).

Important: To reduce risk of cross contamination during the washing procedure avoid back-splashing of liquid while pouring the liquid out of plate! Avoid contacting the walls of the MTP wells with the pipette tips during dispensing the Wash Buffer. If so, change pipette tips!

- Proceed immediately to the detection step.

Detection step

- Prepare 120 µl Assay Reagent (prepared in section 5.) for each well.
- Place the microplate close to or in the reader and add 100 µl of Assay Reagent to each well.

Important: Use a multi-channel pipette or a dispensing pipette in order to reduce the hands-on time.

- Close the reader and wait 1 min to allow the temperature to adjust.
- Read fluorescence signals at time point zero (first reading).
- Incubate the plate at 37°C (incubator or fluorescence reader).

Important: Do not shake the microtiter plate in the fluorescence reader.

- Read fluorescence signals after 90 minutes (second reading).

Important: Longer reaction time may increase sensitivity of test.

6.7 Standard Curve analysis using 4-Parameter Logistic Regression Model

Data processing

- Subtract zero minute values from 90 minute values
- Calculate standard curve according to the following equation:

  Y = (A-D)/(1+(X/C)^B)+D
  with a fit weight: $1/y^2$

- Calculate the sample EU/ml values according to the standard curve parameters
- Calculate regression coefficient (r values should be above 0.98)
- The back calculated value of the 50 – 0,05 EU/ml standard should be in the range of 60 % to 140 %

6.8 Standard Curve analysis using Linear Regression Model

Data export

- Export data (time point zero and time point 90 minutes) into a spreadsheet program

Zero correction

- Subtract time point zero data from time point 90 minute data.
- Calculate the mean value of the zero standard (blank).
- Calculate the mean values of the duplicates of standards and samples.
- Subtract the mean value of the zero standard from the mean values of standards/samples.
- Calculate the logarithm of rfu-values and of the concentration of the standards (EU/ml).

Standard curve

- Plot the standard curve (log(EU/ml) vs. log(rfu)).
- Calculate function by fitting to a linear equation.

  Y = A+ BX

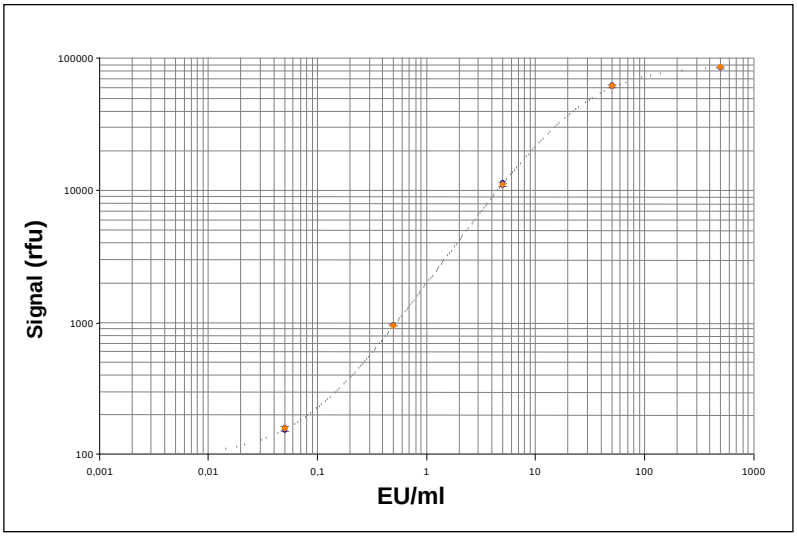
- Calculate regression coefficient (r should be above 0.98).

Sample values

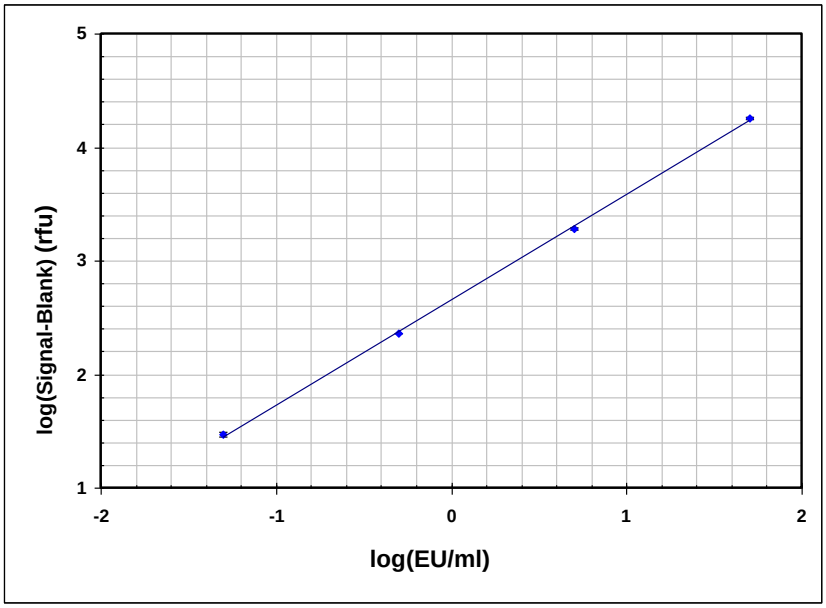
- Calculate EU/ml values of samples on the basis of the linear equation.
- Multiply results with the dilution factor of sample.

6.9 Typical Standard Curves

1. EndoLISA® standard curve (4-Parameter logistic non-linear regression model)
Fig.1



2. EndoLISA® standard curve (linear regression model)
Fig.2



6.10 Influencing Parameters and Limitations

Temperature	The recommended temperature to perform EndoLISA® is 37°C. All reagents should be used at room temperature. LPS binding can be performed at room temperature for 2-6 hours or overnight. For the detection reaction the temperature of 37°C is mandatory.
Agitation	Shaking at 300-500 rpm substantially enhances binding kinetic and to a less extent the kinetic of substrate reaction.
pH	The LPS binding step is not influenced in the pH range between 4.0 and 9.0. If the buffer capacity of the sample is moderate, the Binding Buffer is sufficient to keep the optimal working range. Samples with extreme pH values have to be neutralized before testing.
Salt concentration	Total salt concentration in a sample should not exceed 1 M. In case of higher concentration dilution is required.
Detergents	Detergents at high concentrations (> 0.1%) may interfere with the binding step. Interference can be checked by spiking experiments.
Chelating agents	Chelating agents (e.g. EDTA, EGTA) in the sample should not exceed 0.1 mM. If higher concentrations are present, dilution is required or the chelating agent has to be neutralized with magnesium. Citrate is tolerated up to 5 mM.
Chaotropic agents	High concentrations of chaotropic agents do not interfere with the EndoLISA. For example, guanidine hydrochloride and urea are tolerated up to 1 M and 6 M, respectively
Organic solvents	Interference of organic solvents has to be tested. DMSO is tolerated up to 10%. Methanol, ethanol and 2-propanol are tolerated up to 20%, 30% and 20% respectively.

7. Experienced User Protocol

For first time users it is recommended to refer to the more detailed Assay Protocol (section 6).

1. Getting started: Ensure that all reagents have reached room temperature (20-25°C) before use.

2. Reconstitution of Endotoxin Standard: Dissolve the Standard in Water (endotoxin-free; bottle 2) with the volume indicated on the bottle. Vortex vigorously for at least 10 minutes.

   Note: Endotoxin concentration of the stock solution is 500 EU/ml

   Important: Also vortex your samples, to ensure proper homogeneity.

3. Dilution: Dilute Endotoxin Standard for the standard curve. A final volume of 100 µl is needed for each well. Keep duplicate determinations in mind. Recommended dilution factor is 10 (50 EU/ml; …; 0,005 EU/ml). Vortex for 2 min, before preparing the next dilution step.

   Optional: Samples may require dilution (for more information see section 6.4).

   After your dilution series is complete, vortex again for 10 min.

4. MTP: Select the required number of strips and fix them into the frame.

5. Filling of MTP: Pipette 100 µl of each preparation into the respective wells. Duplicate determinations are recommended.

   Note: Include blank control

   Optional: Spiking of the samples: To ensure measurability of endotoxins in the sample add 10 µl of the 50 EU/ml endotoxin dilution to 100 µl sample.

   Cover the plate with the lid and mix for 1 min.

6. Binding step: Add 20 µl of 6x Binding Buffer to each well of standard and sample. Seal the wells with cover foil and incubate plate at 37°C for 90 minutes at 450 rpm.

7. Start reader: Start the fluorescence reader in time in order to reach the working temperature of 37°C.

8. Washing step: Pour out the liquid into a basin. Wash each well with 3x 150 µl Wash Buffer.

   Important: Do not knock the plate on the bench in any case. Carefully pat the plate dry on a paper towel (top down).

9. Detection step: Prepare 120 µl Assay Reagent for each well by combining 8 parts of Assay Buffer, 1 part of Substrate and 1 part of Enzyme. Mix carefully - do not vortex. Add 100 µl of Assay Reagent to each well.

10. Reader: Measure fluorescence signals at time point zero (first reading). Incubate the plate at 37°C (incubator or fluorescence reader). Measure fluorescence signals after 90 minutes (second reading).

8. Test Principle

Endotoxin

Endotoxins are bacterial cell wall constituents which are recognized by the human immune system and triggers severe physiological reactions. The main endotoxin of gram-negative bacteria is lipopolysaccharide (LPS). LPS is composed of a conserved part (lipid A + conserved core carbohydrate structure) and a highly variable part (O-antigen).

Phage binding proteins

Certain receptor proteins from bacteriophages specifically bind to the conserved carbohydrate structures of lipid A. Such proteins are used to immobilize LPS to the surface of the EndoLISA® assay plate.

Removal of interfering matrix

Existing endotoxin assays (LAL-assays) are in principal highly specific and sensitive. However, as they are homogenous assays, their functionality is often disturbed by interfering substances included in a sample. Therefore dilution of the sample is required at the expense of sensitivity. With immobilizing LPS and introducing a washing step, the interfering matrix is removed in EndoLISA®, without necessity for extensive dilution.

Recombinant Factor C (rFC)

In blood cells of horseshoe crabs, the amebocytes, a coagulation cascade has been evolved to resist infections caused by gram-negative bacteria. The principal receptor of this proteolytic cascade is a protein named Factor C. Factor C is a zymogene (precursor of a protease) that becomes activated by lipid A. In combination with a synthetic fluorescence substrate, a preparation of Factor C is used for detection of the immobilized LPS. EndoLISA® uses a recombinant Factor C (rFC) which is not derived from the blood of the horseshoe crabs.

Test Principle EndoLISA® - Schematic Overview

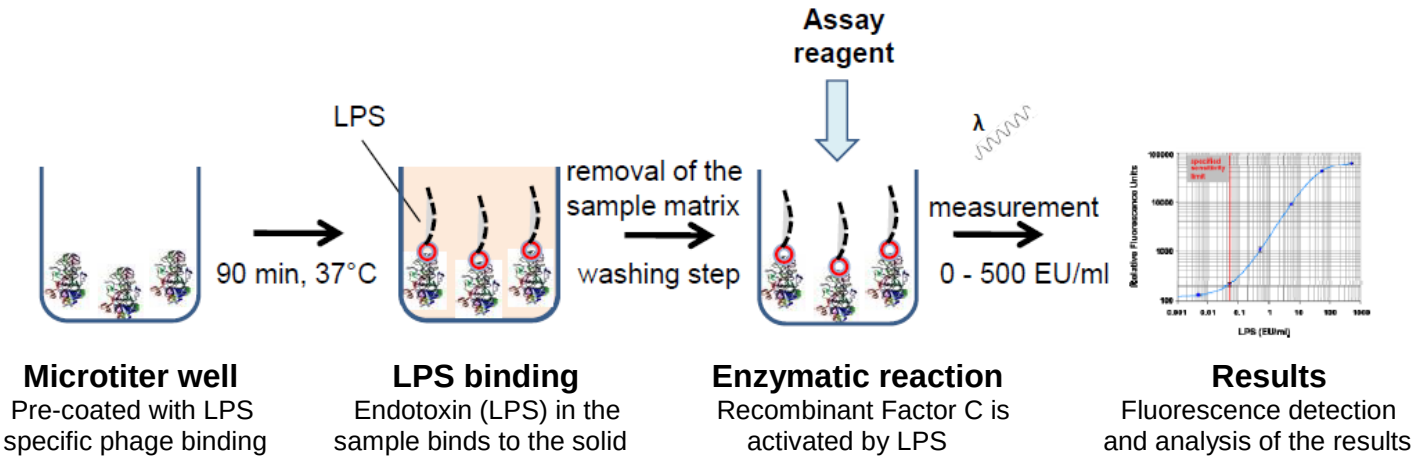


Fig.3 Principle of EndoLISA® test for endotoxin detection. Endotoxin binds to the LPS specific phage binding protein of the pre-coated microtiter plate. Subsequently the sample matrix is removed by a washing step. The assay reagent contains recombinant Factor C (rFC) and a substrate. LPS binding activates the recombinant Factor C (rFC) and the active form of the enzyme modifies the substrate and results in the generation of a fluorogenic compound. After addition of assay reagent to the samples, fluorescence detection is performed in a fluorescence reader. The endotoxin concentration of the samples is determined by standard curve analysis.

9. Trouble Shooting Guide

Observation	Possible Cause		Measure
No signal at all	▪ Wrong instrument settings	⇒	Check instrument parameter
	▪ Lamp defect	⇒	Change lamp
	▪ Pipetting error	⇒	Check reagents, repeat assay
	▪ Incubation temperature much to high or much to low	⇒	Check temperature setting
	▪ Substrate missing	⇒	Prepare the Assay Reagent as described in section 5 Reagent Preparation
No signal with individual samples	▪ Pipetting error (no standard or sample pipetted; no Sample Buffer added)	⇒	Repeat assay
	▪ Interfering ingredients	⇒	Spike control; dilute sample 1:10
	▪ Inappropriate pH	⇒	Check pH; neutralize sample
Low signal level	▪ Wrong sensitivity adjustment (gain)	⇒	Adjust sensitivity
	▪ Reader defect (e.g. optics)	⇒	Run instrument check
	▪ Incubation temperature to high/to low	⇒	Check temperature
	▪ Evaporation during binding step	⇒	Seal wells with cover foil
	▪ Kit damage (shipment or storage)	⇒	Check storage conditions and package material; contact technical service
	▪ Kit or working solutions expired	⇒	Use new kit or fresh reagents
	▪ Inappropriate emission wavelength or band	⇒	Emission filter should not be above 440 nm ; band should be 20-40 nm
	▪ Enzyme missing	⇒	Prepare the Assay Reagent as described in section 5 Reagent Preparation
	▪ Mixing of the standard not appropriate	⇒	For reconstitution of the standard vortex 10 minutes at 1400 rpm; for the preparation of the standard-dilution vortex 2 minutes between each dilution step
	▪ Liquid not removed completely after the washing step	⇒	Pour out the liquid by rapidly inverting the plate and dashing the liquid into a basin
High background signal in standards and negative control	▪ LPS contamination of assay components (e.g. Water)	⇒	Use fresh reagents
	▪ LPS contamination of vials or pipette tips	⇒	Use different lot of vials and pipette tips; switch to glass vials or change supplier
	▪ Inappropriate excitation wavelength or band	⇒	Excitation filter should not be below 360 nm; band 10-20 nm
	▪ Cross contamination	⇒	When removing the liquid during the washing step, pour out the liquid by rapidly inverting the plate and dashing the liquid into a basin
High well-to-well variation	▪ Temperature gradient (incubator, reader)	⇒	Change incubator
	▪ Pipette damage	⇒	Calibrate pipettes
	▪ Plate has been knocked on the bench after the washing procedure	⇒	Carefully pat dry the plate on a paper towel
	▪ Mixing not appropriate	⇒	Vortex sample dilutions and standard dilutions vigorously for 2 minutes at 1400 rpm
Invalid spike control	▪ Interfering ingredients	⇒	Dilute sample 1:10
	▪ Inappropriate pH	⇒	Check pH; neutralize sample
Condensate on cover foils	▪ Heating from bottom; incubator with uneven temperature distribution	⇒	To some extent without negative influence; use different type of incubator

10. Technical Support

EndoLISA® has been developed and is distributed by Hyglos GmbH, Germany. EndoLISA® is manufactured by MicroCoat GmbH, Germany.

Internet

Visit the EndoLISA® website at www.endolisa.com for:

- Technical resources; including manuals, application notes, Certificates of Analysis, Material Safety Data Sheets, FAQ, software templates and references.
- Complete technical service contact information
- Access to price lists and ordering forms
- Additional product information and special offers

Contact us

For more information or technical assistance, call, write, fax or e-mail.
Additional international distributors are listed on our website www.endolisa.com

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11. Legal Statements and Regulatory Information

Guidelines for sample testing in regulated environments can be found in the European and US Pharmacopoeia (Chapter 4.6.14 in combination with chapter 5.1.10 and chapter 85, respectively).

Trademarks

EndoLISA®, EndoZyme®, EndoTrap® and EndoGrade® are registered trademarks of Hyglos Invest GmbH
FLx800™ and Gen5™ are trademarks of BioTek Instruments

Patent information

Various components of EndoLISA® are protected under the following patents: US7858299, US7585620, EP1516188, EP1516188, CN1662816, CN100343671, AU2003250270, CA100343671, JP4659453, KR101036456, PL209568, RU2344425, EP1844333, US8003313, US8329393, EP1695085, DE10360844, AU2004303928, EP1695085 and US8394597.

Parts of EndoLISA® are licensed under the following patents: US6849426, AU2002330860, CN100390193 and JP5039729.

12. Related Products

EndoZyme® Endotoxin Detection Assay

- **EndoZyme®** - Homogeneous Recombinant Factor C (rFC) Endotoxin Detection Assay

EndoTrap® Endotoxin Removal

- **EndoTrap® blue** - for use with e.g. HEPES, TRIS, MOPS, MES, PIPES buffered samples
- **EndoTrap® red** - for use with e.g. PBS buffered samples
- **EndoTrap® HD** - for evaluation and production processes in biotech/pharma

As a complete workflow solution Hyglos recommends the combination of EndoTrap® red and EndoLISA® since there solid phases differ.

EndoGrade® Endotoxin-free Accessories

- **EndoGrade® Glass Test Tubes** - endotoxin-free borosilicate glass test tubes with screw cap

EndoGrade® Endotoxin-free Reagents

- **EndoGrade® Ovalbumin** - purified ovalbumin for immunology research