EndoLISA®

Endotoxin Detection Assay based on ELISA-technology

Fluorescence microplate assay using a bacteriophage-derived capture molecule as solid phase and Recombinant Horseshoe Crab Factor C for detection.

Package Insert EndoLISA®

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1. General Information

1.1 Intended Use

Intended use

EndoLISA® is intended for quantitative determination of endotoxin (chemically lipopolysaccharide, LPS) in pharmaceutical end-products, in-process control and research samples, and medical device testing. EndoLISA® is particularly suited for complex samples.

1.2 Test Principle

EndoLISA® is an ELISA-like heterogeneous enzymatic assay which uses a bacteriophage-derived capture molecule as solid phase for removal of interfering substances and subsequent detection with Recombinant Horshoe Crab Factor C (rFC) in combination with a fluorogenic substrate.

Endotoxin

Endotoxins are bacterial cell wall constituents which are recognized by the human immune system and trigger 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 endotoxin in the wells of the EndoLISA® Assay Plate.

Removal of interfering substances

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 the endotoxin binding and wash step, the interfering matrix is removed in the EndoLISA® assay prior to detection, without necessity for extensive dilution.

Recombinant Factor C (rFC)

In blood cells of horseshoe crabs, the amebocytes, a coagulation cascade has 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 fluorogenic substrate, a preparation of Factor C is used for detection of the immobilized LPS. EndoLISA® uses a synthetic, recombinant Factor C (rFC) which is not derived from the blood of the horseshoe crabs.

Test Principle EndoLISA® - Schematic Overview

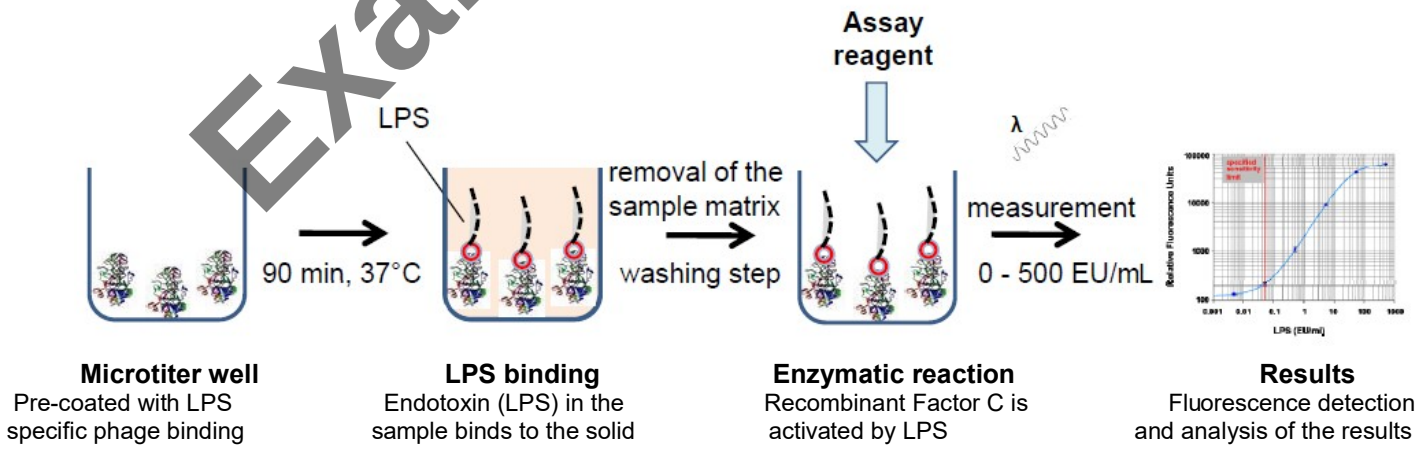


Fig.1. Principle of the EndoLISA® test for endotoxin detection. Endotoxin binds to the endotoxin-specific phage binding protein of the pre-coated microtiter plate. Subsequently the sample matrix is removed by a wash step. The assay reagent contains recombinant Factor C (rFC) and a substrate. Endotoxin binding activates the recombinant Factor C (rFC) and the active form of the enzyme modifies the substrate and results in the generation of a fluorescent 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.

1.3 Specifications

Assay range 0.05 to 500 EU/mL

Quantitation limit 0.05 EU/mL

Assay time 90 minutes binding (incubation) + 90 minutes detection time

2. Kit Components

Number of tests The kit contains reagents for 192 tests.

Kit components

Component	Container	Content	Description
1 EndoLISA® Microtiter plate (**EndoLISA MTP**)	Aluminium bag	2 plates	Pre-coated microplates; a total of 12 modules with 16 wells each.
2 Water (**WEF**)	Plastic bottle, blue cap	2 x 100 mL	Water, free of detectable levels of endotoxin, for reconstitution of the standard and, dilution of standard and samples.
3 Binding Buffer 6x (**BB**)	Glass bottle, white cap	2 x 2.5 mL	Binding buffer, 6-fold concentrated, ready-to-use.
4 Endotoxin Standard (*E. coli* O55:B5) **(CSE)**	Glass bottle, red cap	2 bottles	Endotoxin standard, lyophilized, containing approx. 1000 EU of LPS from *E. coli* O55:B5.
5 Wash Buffer (**WB**)	Plastic bottle, yellow cap	2 x 75 mL	Wash buffer; ready-to-use.
6 Enzyme (**ENZ**)	Plastic bottle, transparent cap	2.5 mL	Enzyme solution for the detection of LPS, 10-fold concentrated. This kit component contains products of animal origin (Bovine Serum Albumin).
7 Substrate (**SUB**)	Brown plastic bottle, brown cap	2.5 mL	Fluorescence substrate, 10-fold concentrated.
8 Assay Buffer (**AB**)	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.

3. Warnings and Precautions

Warning: EndoLISA® is not intended for use with clinical samples or for diagnosis of human or animal disease.
For professional use only.
The kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).

Endotoxin-free conditions All materials used, such as containers or pipette tips, should be free of detectable levels of endotoxin. For preparing sample and standard dilutions, glass test tubes are recommended, since endotoxin may adhere to hydrophobic plastic surfaces.

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. Additional Reagents, Equipment, Instrumentation and Software Required

Reagents

- If required: Endo-RS® Endotoxin Recovery Kit (Surfactant) - Ref 609065, for demasking endotoxin in biopharmaceutical formulations containing surface active agents.

Equipment and Disposables

- Pipettes
- Multi-channel pipette
- Dispensing pipette
- Pipette tips, endotoxin-free
- Dispensing pipette tips, endotoxin-free
- Glass test tubes, endotoxin-free - (e.g.: EndoGrade® Glass Test Tubes- Ref 800050)
- Reagent reservoir, endotoxin-free

Instruments

Vortex-type mixer

0-1500 rpm
To reconstitute the Endotoxin Standard (CSE), mix thoroughly by vortexing at 1400 rpm for 10 minutes. Sample dilutions and standard dilutions should be mixed vigorously for 2 minutes. This is optimally achieved by using a multi-tube Vortex-type mixer.

Incubator / plate shaker

Incubator 37°C
Plate shaker, 0-800 rpm
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, temperature controlled

Fluorescence microplate readers from different suppliers may be used for reading of EndoLISA® results.

Instrument settings:	Temperature	37°C
	Excitation filter (nm/band)	380
	Emission filter (nm/band)	445
	Reading orientation	From top
	Readings per well	Minimum 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 specifically. **Do not use automatic gain adjustment**, since it may change the gain throughout the measurement. 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.

Calculation software

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

5. Reagents Storage and Preparation

Storage and stability

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

Use of kit components, stability and storage conditions

Reagent	Preparation	Stability and storage conditions of working solutions
1 EndoLISA® Microtiter plate (**EndoLISA MTP**)	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 (**WEF**)	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
3 Binding Buffer 6x (**BB**)	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
4 Endotoxin Standard (*E. coli* O55:B5) **(CSE)**	The reconstitution volume is indicated on the label; resolve lyophilized standard with water (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 (**WB**)	Ready-to use	Stable until expiry date of the kit when stored at 2-8°C
6 Enzyme (**ENZ**)	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
7 Substrate (**SUB**)	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
8 Assay Buffer (**AB**)	For Assay Reagent preparation	Stable until expiry date of the kit when stored at 2-8°C
9 Cover Foil	Self-adhesive foils for single use; cut off the required size and remove protective sheet	Stable

Reagents to be prepared from kit components

Reconstitution of Endotoxin Standard (CSE):

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

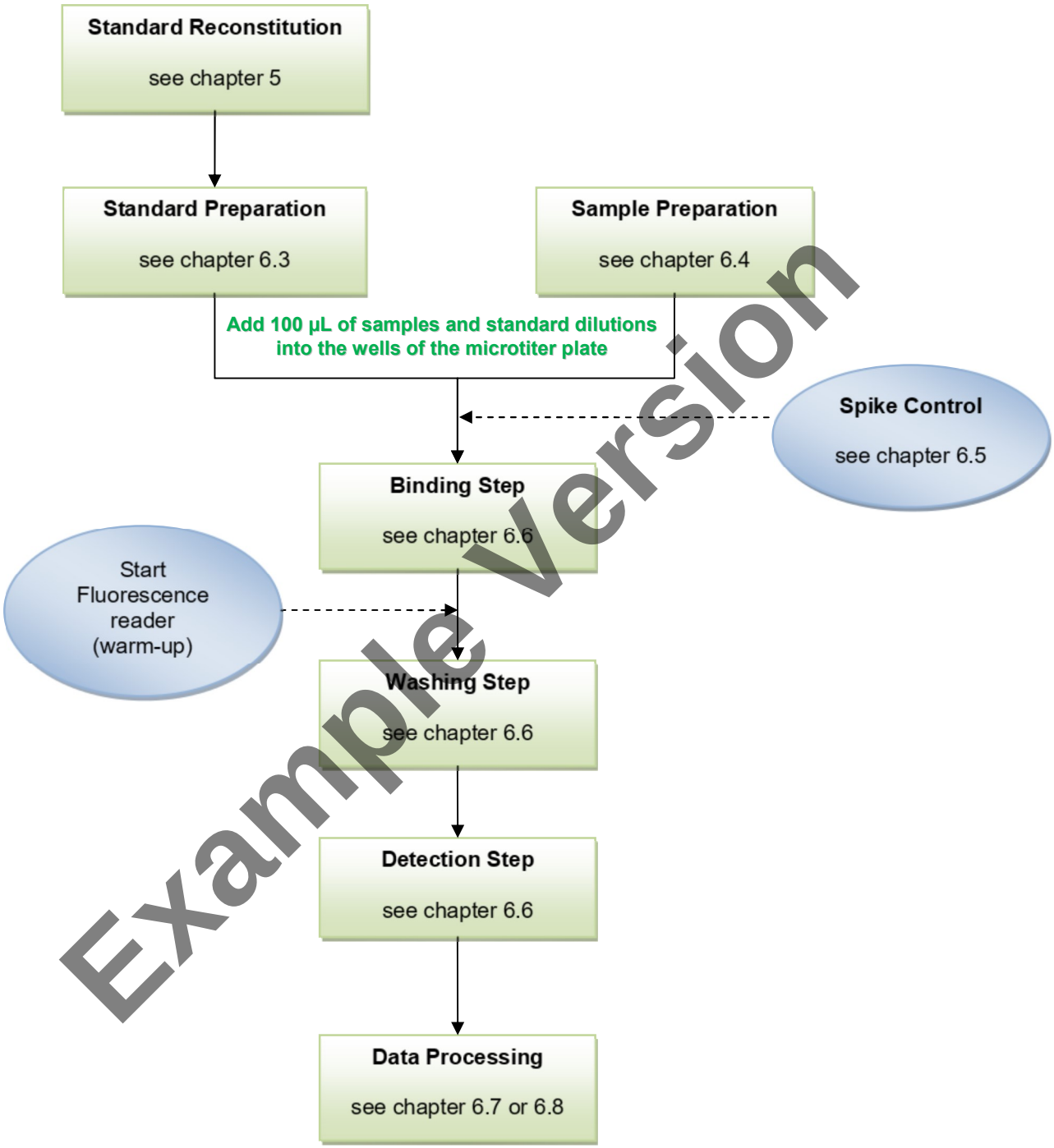
  Important: Use new pipette tips for every pipetting step to avoid contamination of the water.

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

Assay Reagent: Prepare the Assay Reagent freshly immediately before use (see chapter 6.6, Assay Procedure, for quantities).

6. Assay Protocol

6.1 Overview Assay Procedure



Watch the EndoLISA® Video User-Guide on-line for step-by-step guidance:

www.endolisa.com

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.

6.3 Standard Preparation

Serial dilution of Endotoxin Standard - CSE:

- The reconstituted CSE (4) has a concentration of 500 EU/mL (= first standard concentration). For the reconstitution of CSE, 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 water (2) into each tube prepared for the dilution series.
- Add 100 µL of the reconstituted CSE (4) to prepare the second standard. Close the vial and mix thoroughly by vortexing 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 water (2) as blank (negative control).
- Standard dilutions are stable for 8 hours 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	+	+

6.4 Sample Preparation

Sample preparation/ sample dilution

Many samples can be analyzed undiluted with the EndoLISA® assay, but certain substances may require dilution (see section 6.10). In such cases, a sample dilution of 1:10 in water (2) is recommended.

- For sample dilution, use glass test tubes free of detectable levels of endotoxin.
- Pipette 400 µL of endotoxin-free water (2) into an empty vial and add 100 µl sample. Vortex for 2 minutes.

6.5 Spike Control

Spiking of samples

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

Spike material

The Endotoxin Standard (CSE) provided with the kit can be used for spike control.

Spike concentration

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

Example:
- Add 10 µL of the 50 EU/mL standard to a sample (spike = 5 EU/mL).

Validity criteria

A result is considered valid, if the spike recovery is in the range of 50% to 200%. Samples with insufficient spike recovery have to be tested with higher dilution.

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 the 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

Assay Reagent: Required amounts are indicated in the table below. Combine 8 parts of Assay Buffer (8), 1 part of Substrate (7) and 1 part of Enzyme (6). Mix carefully - do not vortex.

Prepare 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 mLfor 96 reactions	9.6 mL	1.2 mL	1.2 mL

Filling of MTP

- Select the required number of strips needed for the run and store unused strips in the resealable aluminium bag.
- 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 samples and standard dilutions are vortexted prior to use.

Binding step

- Add 20 µL of Binding Buffer (3) to each well of standard and sample. Pipette carefully and use fresh tips to avoid cross-contamination.
- Seal the wells with cover foil.
- Incubate the plate at 37°C for 90 minutes with continuous mixing at 450 rpm.

Important: Highly complex or concentrated samples may require a prolonged binding step (90 min to 18h) since endotoxin 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 the risk of cross contamination during the washing procedure avoid back-splashing of liquid while pouring the liquid out of the plate! Avoid contacting the walls of the MTP wells with the pipette tips during dispensing of the Wash Buffer. If so, change pipette tips!

- Proceed immediately to the detection step.

Detection step

- Prepare sufficient amount of Assay Reagent (see above). Place the microplate close to or in the fluorescence 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 the 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 the 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 the regression coefficient (r values should be at least 0.980)
- The back calculated values 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 at least 0.980).

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

Fig. 2. EndoLISA® standard curve (4-Parameter logistic non-linear regression model)

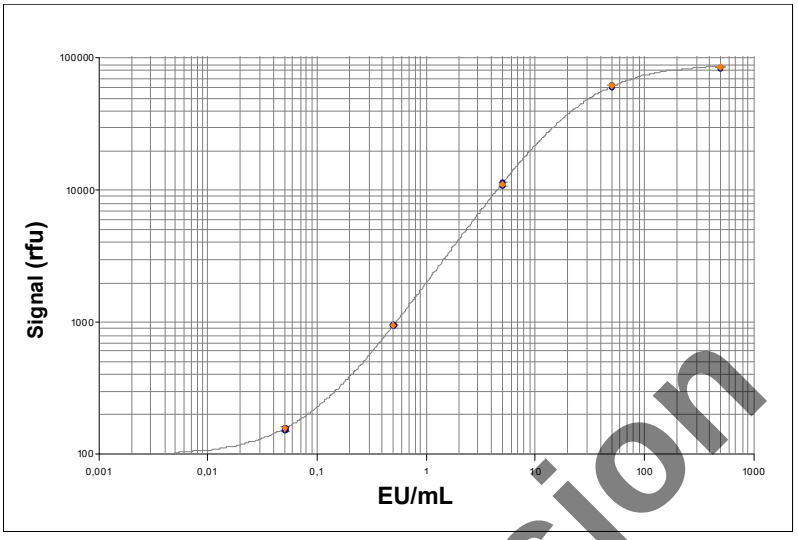
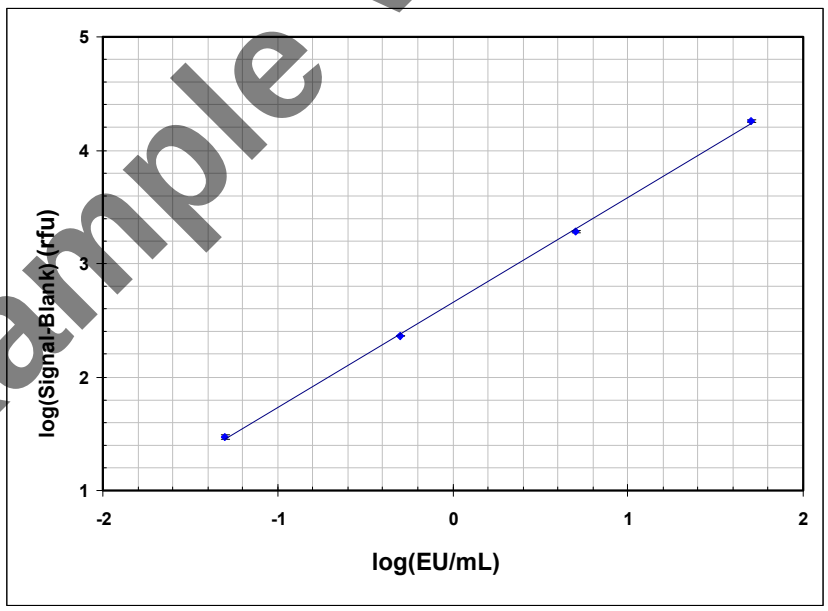


Fig. 3. EndoLISA® standard curve (linear regression model)



6.10 Influencing Parameters and Limitations

Temperature The recommended temperature to perform EndoLISA® is 37°C. All reagents should be used at room temperature. The endotoxin binding step 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 endotoxin binding step is functional 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 and endotoxin masking can be checked by hold time 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.

Masked Endotoxin Masked endotoxins in biopharmaceuticals are not detectable in commonly used testing procedures without prior demasking. The Endo-RS® kit is intended for demasking samples in which masking is mainly triggered by non-ionic surface active agents (surfactants) such as Polysorbate 20, Polysorbate 80 or Triton X-100.

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 CSE 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 CSE 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).
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. Waste Disposal

Unused reagents may be considered as non hazardous waste and disposed of accordingly. Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

9. Quality Control

EndoLISA® has been designed and developed to meet the strictest quality requirements. The results of quality control are given on the quality control certificate available from our website (www.hyglos.com).

10. Trouble Shooting Guide

Observation	Possible Cause		Measure
No signal at all	▪ Wrong instrument settings	⇒	Check instrument parameters
	▪ Lamp defect	⇒	Change lamp
	▪ Pipetting error	⇒	Check reagents, repeat assay
	▪ Incubation temperature too high or much too 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 too high/too 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 completely removed 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

11. Legal Statements and Regulatory Information

Recombinant Factor C is included as an alternative method in the **European Pharmacopoeia Chapter <5.1.10>**, Section 12-2. Chapter <5.1.10> also describes the solid phase included in the EndoLISA assays in Section 9: Removal of interfering factors. In the **FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers**, Recombinant Horseshoe Crab Factor C is included as an alternative method in Section 5. Guidelines for validation of alternative methods can be found in the USP Chapter <1225> and Ph. Eur. Chapter <2.6.14>.

Patent information

Various components of EndoLISA® are protected under the following patents: US7858299, US7585620, EP1516188, CN1662816, CN100343671, AU2003250270, CA2490467, JP4659453, KR101036456, PL209568, RU2344425, EP1844333, US8003313, US8329393, EP1695085, DE10360844, AU2004303928, CA2595476 and US8394597.

Parts of EndoLISA® are licensed under the following patents: US6849426, AU2002330860, CN100390193, BR0210681 and JP5039729.

12. Index of Symbols and Abbreviations

Symbol	Meaning
	Catalog number
	Manufacturer
	Date of manufacture
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Do not re-use

Abbreviations used:

CSE	Control Standard Endotoxin
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))
FDA	Food and Drug Administration
LAL	*Limulus amebocyte lysate*
LLOQ	Lower limit of quantification
LPS	Lipopolysaccharide
MTP	Microtiter plate
rFC	Recombinant Factor C
RFU	Relative fluorescence unit
rpm	Revolutions per minute
RSE	Reference Standard Endotoxin
USP	United States Pharmacopeia

13. Limited Warranty

Hyglos / bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, Hyglos / bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the “System”) other than as set forth in the IFU.

14. Revision History

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
	Minor typographical, grammar, and formatting changes are not included in the revision history.

Release date	Part Number	Change Type	Change Summary
2017/01	606168 V2.0	Administrative	*All – change of template*

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