BIOMÉRIEUX

REF 424107 / 424109

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ENDOZYME® II GO STRIPS / ENDOZYME® II GO STRIPS ST
Recombinant Factor C Endotoxin Detection Assay

For microbiological control only

1. Intended Use

Fluorescence microplate assay using the strips prefilled with Control Standard Endotoxin (CSE).

ENDOZYME® II GO STRIPS and ENDOZYME® II GO STRIPS ST are intended for quantitative determination of endotoxin (chemically lipopolysaccharide, LPS) in liquid samples such as water. They can also be used for quantitative determination of endotoxin in pharmaceutical final products, in-process control and medical device testing.

2. Explanation and Principle

This homogeneous enzymatic assay uses the synthetic endotoxin sensor (Recombinant Factor C) derived from the blood clotting cascade of horseshoe crabs in combination with a fluorogenic substrate.

Endotoxins are bacterial cell membrane constituents which are recognized by the human immune system and may trigger severe physiological reactions. A frequently-used synonym for endotoxin is lipopolysaccharide (LPS). LPS is composed of a conserved part (lipid A + conserved core carbohydrate structure) and a highly variable part (O-antigen).

In blood cells of horseshoe crabs such as *Limulus polyphemus* and *Tachypleus tridentatus* (amebocytes), a coagulation cascade has evolved to resist infections caused by Gram-negative bacteria. The principal sensor of this proteolytic cascade is a protein named Factor C. It is a zymogen/proenzyme (precursor of an enzyme, here protease) that is activated by endotoxin.

Recombinant Factor C (rFC), instead of *Limulus* or *Tachypleus* amebocyte lysate (LAL or TAL), is used in combination with a synthetic fluorogenic substrate for detection of endotoxin.

Specifications:

Assay range	0.005 to 5 EU/mL EU = Endotoxin Unit/1 EU = 1 IU (International Unit) 1 EU corresponds to 0.1 ng LPS (Food and Drug Administration (FDA) Reference Standard Endotoxin (RSE) *Escherichia coli* O113 EC-7)
Sensitivity (λ)	0.005 EU/mL
Assay time	60 minutes (0.005 EU/mL) A shorter assay time is possible depending on required sensitivity (see section 7.2).

3. Components

ENDOZYME® II GO STRIPS (REF 424107)

Designation	Container	Presentation	Description
1 **ENZYME**	Plastic bottle Transparent cap	6 x 2.5 mL	Enzyme (rFC) solution. This component contains products of animal origin (Bovine Serum Albumin).
2 **SUBSTRATE**	Brown plastic bottle Brown cap	6 x 2.5 mL	Fluorescence substrate.
3 **ASSAY BUFFER**	Brown plastic bottle Brown cap	12 x 12 mL	Assay Buffer, to be combined with Substrate 2 and Enzyme 1.
4a **CURVE STRIPS**	Aluminium bag	4 plates	Assay plate containing 6 strips pre-loaded with Control Standard Endotoxin (CSE) of *E. coli* O113:H10. For standard curve, blank, one sample and one Positive Product Control (PPC).
4b **SAMPLE STRIPS**	Aluminum bag	8 plates	Assay plate containing 6 strips pre-loaded with Control Standard Endotoxin (CSE) of *E. coli* O113:H10. For four samples and four PPC.
7 **SCAFFOLD**		1 scaffold	
1 package insert downloadable from www.biomerieux.com			

ENDOZYME® II GO STRIPS ST (REF 424109)

Designation	Container	Presentation	Description
1 **ENZYME**	Plastic bottle Transparent cap	6 x 2.5 mL	Enzyme (rFC) solution. This component contains products of animal origin (Bovine Serum Albumin).
2 **SUBSTRATE**	Brown plastic bottle Brown cap	6 x 2.5 mL	Fluorescence substrate.
3 **ASSAY BUFFER**	Brown plastic bottle Brown cap	12 x 12 mL	Assay Buffer, to be combined with Substrate 2 and Enzyme 1.
4a **CURVE STRIPS**	Aluminum bag	12 plates	Assay plate containing 6 strips pre-loaded with Control Standard Endotoxin (CSE) of *E. coli* O113:H10. For standard curve, blank, one sample and one Positive Product Control (PPC).
7 **SCAFFOLD**		1 scaffold	
1 package insert downloadable from www.biomerieux.com			

4. Warnings and Precautions

- **The assay is not intended for use with clinical samples or for diagnosis of human or animal disease.**
- **For microbiological control only.**
- **For professional use only.**
- This 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).
- Each strip is for single use and should not be reused. Scaffolds can be reused.
- All materials used, such as containers or pipette tips, should be free of detectable levels of endotoxin and interference.
- Glass test tubes are recommended for the preparation of the sample since endotoxin may adhere to hydrophobic plastic surfaces.
- After standard cleaning procedure, glass should be heated at +200°C for 4 hours. Use aluminum caps or aluminum foil to seal openings.
- Plastic material may be treated with 1 M NaOH for 6-12 hours. Afterwards, rinse with a large volume of endotoxin-free water and let it air dry. Final pH of the rinsing water should be neutral.
- Samples should be stored refrigerated or frozen.
- Treat samples carefully in order to avoid microbial or endotoxin contamination.
- Do not mix reagents (or disposables) from different lots.
- Do not use reagents after the expiration date indicated on the label.
- The test must be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

5. Reagents, Materials, and Disposables Required but Not Provided

- Pipettes.
- Multichannel pipette or dispensing pipette.
- Endotoxin-free and without interference pipette tips.
- Endotoxin-free and without interference glass test tubes (such as ENDOGRADE® Glass Test Tubes - Ref. 800050).
- Endotoxin-free water (such as ENDOGRADE® Water - Ref. 607100).
- Vortex-type mixer (0-1,500 rpm).
- Incubator (optional).
- Fluorescence microplate reader: Fluorescence microplate readers from different suppliers may be used to read the results.

Instrument Settings	
Temperature	+37°C
Excitation (nm)	380
Emission (nm)	445 (445 is the optimum wavelength. A 440 filter can be used.)
Optics position	Top
Readings per well	Minimum 10
Shaking mode	On (Shake for 15 seconds at medium intensity prior to reading Time Point 0)
Sensitivity/PMT gain	To be determined for each instrument and reagent lot (see section 7.1)

- Calculation software.

6. Storage Conditions

Unopened kits are stable at +2°C to +8°C until the expiry date.

For further information on storage and stability of the individual components, please refer to the table below.

ENDOZYME® II GO STRIPS (REF 424107)

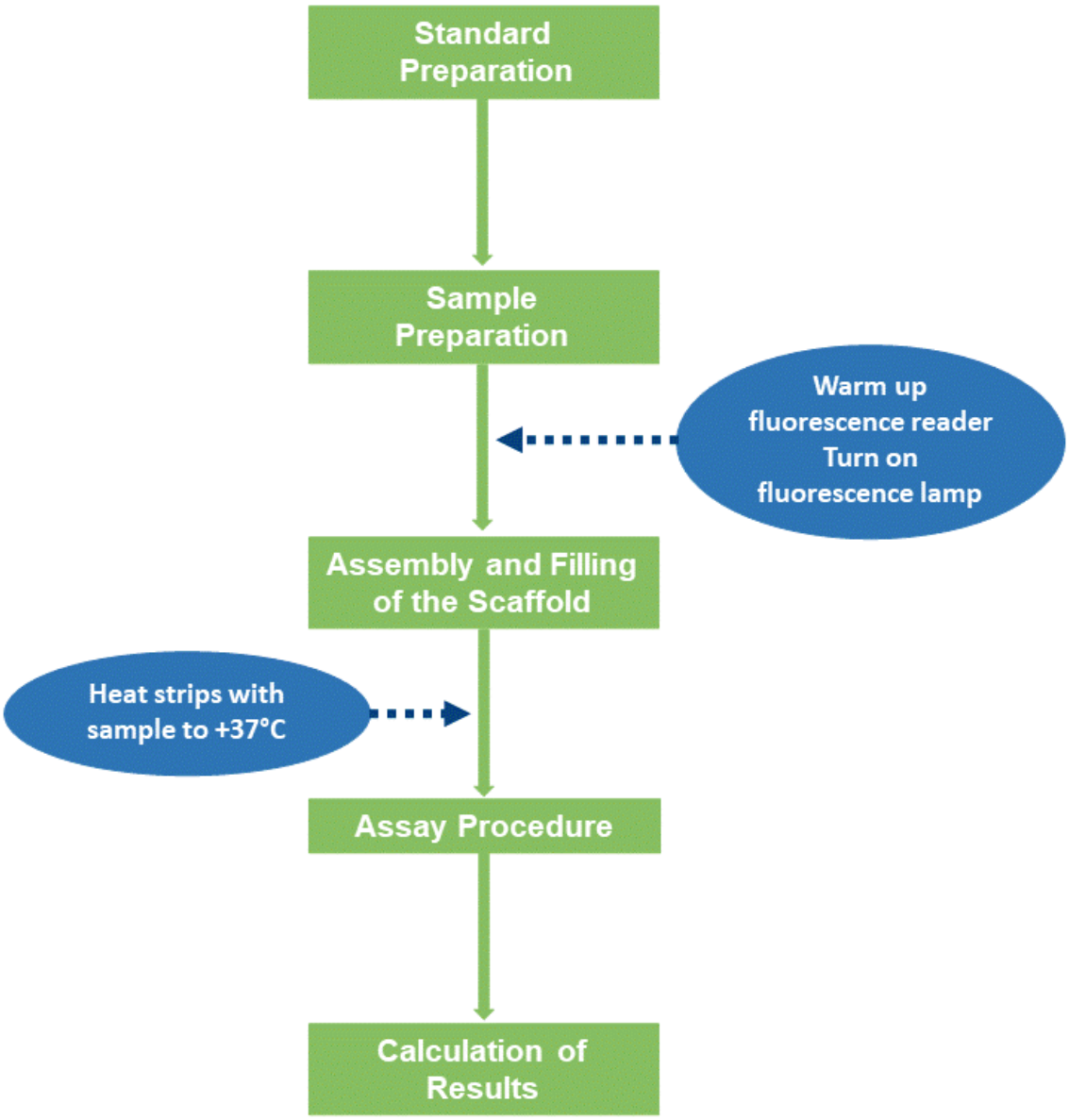
Reagent	Preparation	Stability and Storage Conditions
1 **ENZYME**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
2 **SUBSTRATE**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
3 **ASSAY BUFFER**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
4a **CURVE STRIPS**	Ready-to-use. For Standard Curve preparation and for application of one sample	Stable when unopened until expiry date of the kit. Once opened the strips are stable for 3 months when stored dry at +2°C to +8°C.
4b **SAMPLE STRIPS**	Ready-to-use. For Sample application	Stable when unopened until expiry date of the kit. Once opened the strips are stable for 3 months when stored dry at +2°C to +8°C.

ENDOZYME® II GO STRIPS ST (REF 424109)

Reagent	Preparation	Stability and Storage Conditions
1 **ENZYME**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
2 **SUBSTRATE**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
3 **ASSAY BUFFER**	For Assay Reagent preparation	Stable until expiry date of the kit when stored at +2°C to +8°C.
4a **CURVE STRIPS**	Ready-to-use. For Standard Curve preparation and for application of one sample	Stable when unopened until expiry date of the kit. Once opened the strips are stable for 3 months when stored dry at +2°C to +8°C.

7. Procedure

- Allow components to come to room temperature at +20°C to +25°C before use.
- Be careful not to contaminate the kit components in use.
- Pipette thoroughly to ensure accurate transfer of the small volumes.
- Perform a standard curve in parallel to each test series.
- Perform all measurements at least in duplicates.



7.1. Gain Adjustment

The logarithm of the dRFU correlates with the logarithm of the gain (dRFU = Difference in Relative Fluorescence Units (RFU) between time points of a single measurement). Accordingly, the optimum gain can be determined by analyzing several 0.5 EU/mL replicates.

At the installation of the reader and once per reagent lot:

1. • ENDOZYME® II GO STRIPS (REF 424107): Pipette 100 µL of endotoxin-free water into four PPC wells, such as rows G and H of one sample strip.
   • ENDOZYME® II GO STRIPS ST (REF 424109): Pipette 100 µL of endotoxin-free water into four 0.5 EU/mL wells, using the wells in rows C and H of one curve strip.
2. Put the microplate into the fluorescence reader and warm up the microplate to +37°C.
3. Combine 400 µL of Assay Buffer 3, 50 µL of Substrate 2 and 50 µL of Enzyme 1 in an endotoxin-free and non-interfering reagent reservoir or tube.
4. Mix carefully.

5. Add 100 µL of assay reagent (see Assay Procedure) to each of the previously mentioned four wells.
6. Run the assay according to the Assay Procedure.
7. Calculate the optimum gain:

   Subtract time point zero data from time point 60 minutes data (dRFU) and calculate the mean dRFU for each gain. Optimum gain = current gain * 10^(log(optimum dRFU/current dRFU)/7.2).

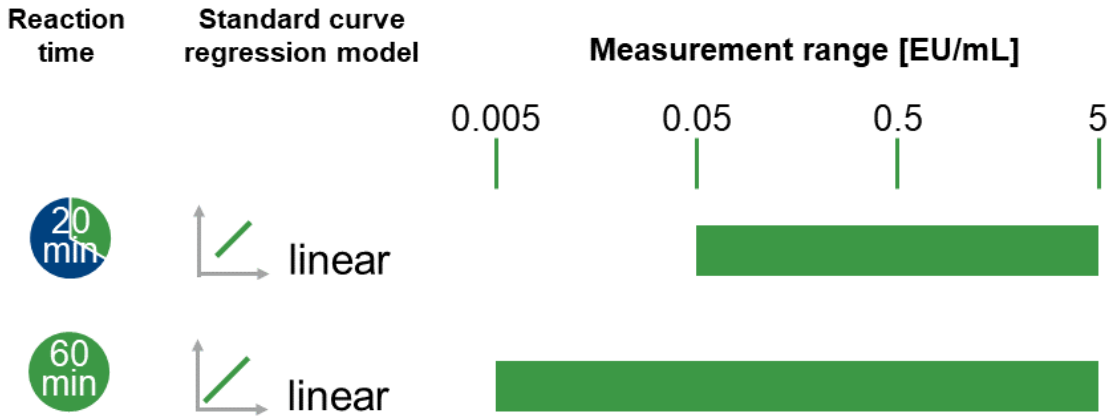
   For linear regression: optimum dRFU = 5% of maximum RFU.

7.2. Standard Preparation

The curve strips **4a** are pre-filled with the following concentrations of CSE from *Escherichia coli* O113:H10 (see Assembly and Filling of the Scaffold for scaffold layout):

- 50 EU/mL
- 5 EU/mL
- 0.5 EU/mL
- 0.05 EU/mL
- 0.005 EU/mL

If a sensitivity of only 0.05 EU/mL is required, the assay run time can be shortened to 20 minutes and 0.005 EU/mL are excluded from the analysis. This has to be validated by the user.



7.3. Sample Preparation

For sample dilution, use endotoxin-free and non-interfering glass test tubes.

For example, a 1:10 dilution should be prepared as follows:

1. Pipette 900 µL of endotoxin-free water into a vial and add 100 µL of sample.
2. Mix using a vortex-type mixer for at least 1 minute.

7.4. Assembly and Filling of the Scaffold

The scaffold can be assembled as per the need of the user before every single test run.

ENDOZYME® II GO STRIPS (REF 424107) :

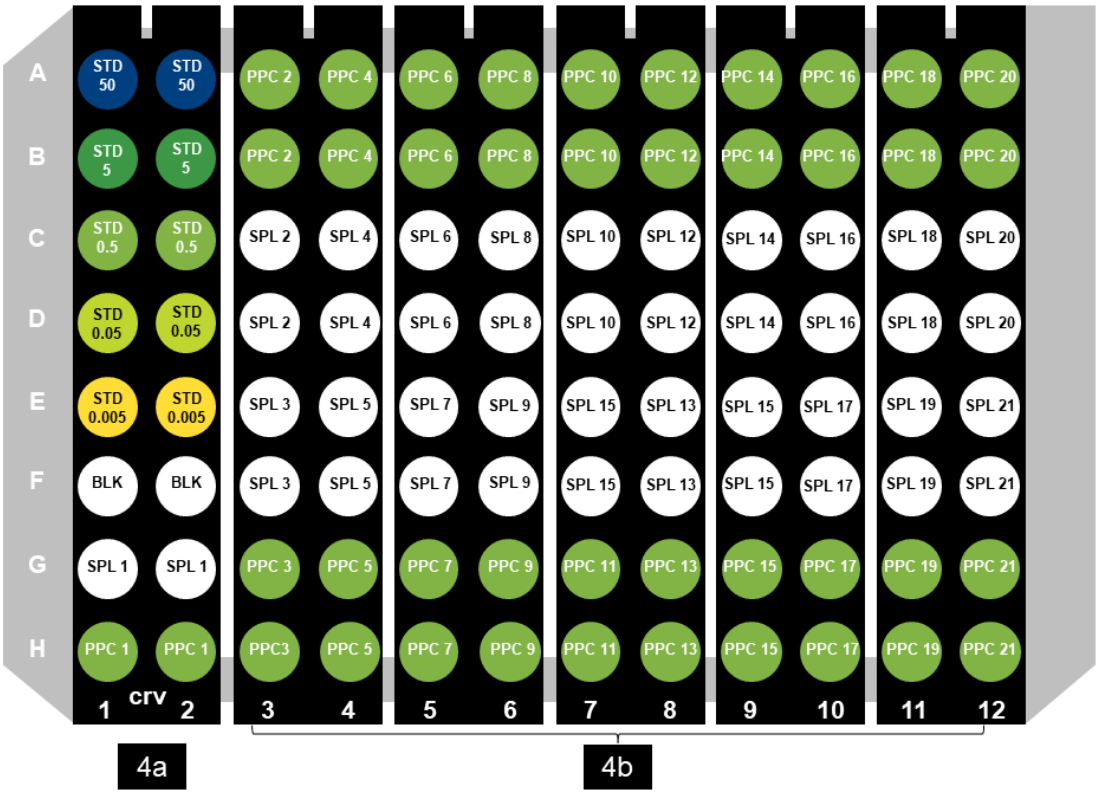
1. Place one curve strip **4a** on an empty scaffold. The strip should click into the correct place.
2. Add one to five sample strips **4b** on the scaffold.

Note: One curve strip allows for testing one sample, each sample strip for testing four samples, respectively. One to 21 samples can be tested on a single scaffold.

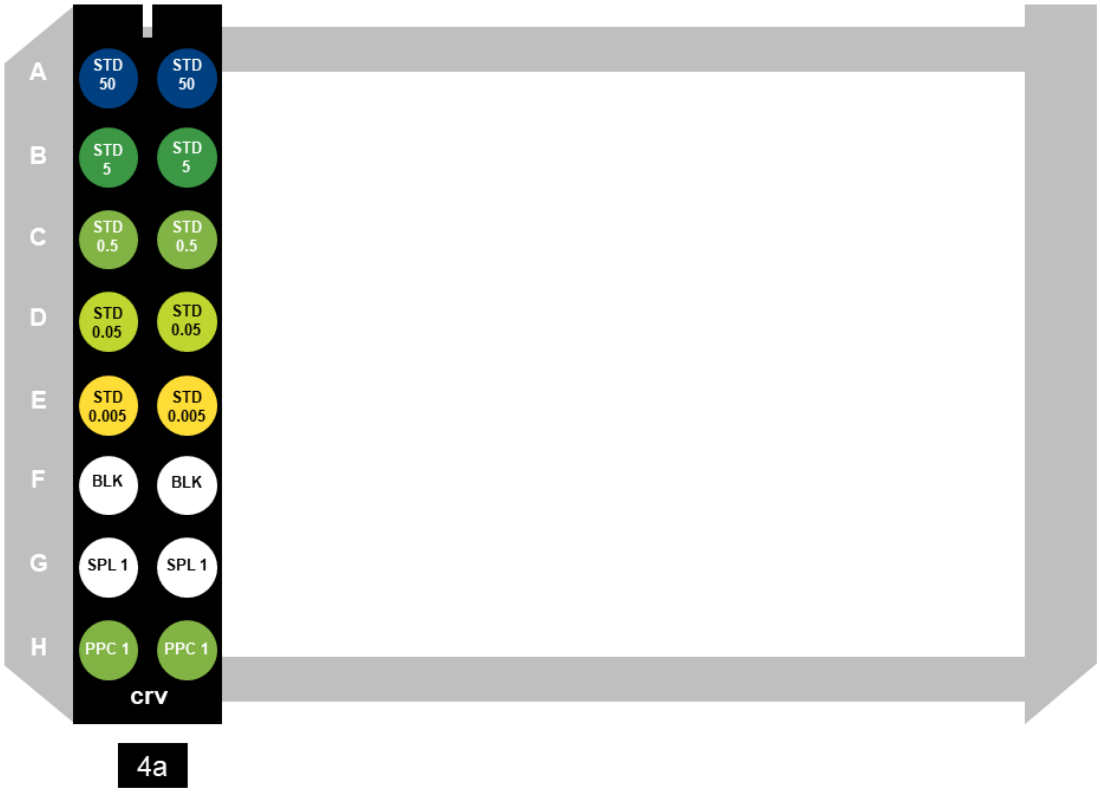
ENDOZYME® II GO STRIPS ST (REF 424109) : Place one curve strip **4a** on an empty scaffold.
Note: One curve strip allows for testing one sample.

Scaffold Layout Examples

ENDOZYME® II GO STRIPS (REF 424107)



ENDOZYME® II GO STRIPS ST (REF 424109)



STD = Control Standard Endotoxin

BLK = blank, empty well

PPC = Positive Product Control (= 0.5 EU/mL)

SPL = sample, empty well

crv = curve

1. Reconstitute CSE and add blank by pipetting 100 µL of endotoxin-free water into wells A1 to F2 of the curve strip **4a**.
2. ENDOZYME® II GO STRIPS (REF 424107): Pipette 100 µL of sample into at least four wells of a sample strip and wells G1 to H2 of the curve strip: two wells without PPC, two wells with PPC.
   ENDOZYME® II GO STRIPS ST (REF 424109): Pipette 100 µL of sample into wells G1 to H2 of the curve strip: two wells without PPC, two wells with PPC.
3. Put the scaffold into the fluorescence reader and warm up the scaffold with samples to +37°C for at least five minutes.

7.5. Spike Control

Spiking of samples should be applied to validate if sample components interfere with the assay and if dilution is required.

7.6. Assay Procedure

Prepare the Assay Reagent freshly immediately before use.

1. Combine eight parts of assay buffer **3**, one part of enzyme **1** and one part of substrate **2** in an endotoxin-free and non-interfering reagent reservoir or tube.

Required Volumes			
Assay Reagent	**Assay Buffer**	**Substrate**	**Enzyme**
2 mL for 1 strip	1.6 mL	0.2 mL	0.2 mL
4 mL for 2 strips	3.2 mL	0.4 mL	0.4 mL
6 mL for 3 strips	4.8 mL	0.6 mL	0.6 mL
8 mL for 4 strips	6.4 mL	0.8 mL	0.8 mL
10 mL for 5 strips	8.0 mL	1.0 mL	1.0 mL
12 mL for 6 strips	9.6 mL	1.2 mL	1.2 mL

   Note: In case of using different volumes, compensate for the dead volume of the pipette.
2. Mix carefully. **Do not mix with a vortex-type mixer.**
3. Add 100 µL of Assay Reagent to each well.
   Note: Use a dispensing pipette or a multi-channel pipette in order to reduce the hands-on time.
4. Close the reader containing the plate and wait one minute to allow the temperature to adjust.
5. Shake for 15 seconds at medium intensity.
6. Read fluorescence signals at time point zero (first reading).
7. Incubate the microplate for the appropriate time depending on the sensibility required (60 minutes or less) at +37°C (incubator or fluorescence reader).
8. Read fluorescence signals (second reading).

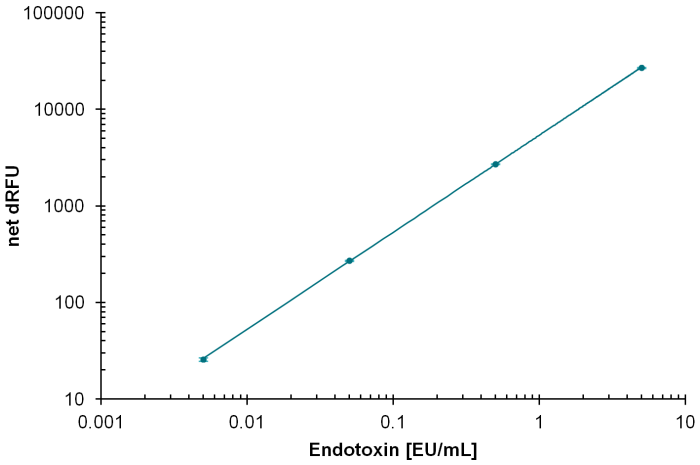
8. Calculation of Results

8.1. Linear Regression Model of Standard Curve

1. Collect raw data values of the initial reading (time point zero) and the final reading.
2. Subtract time point zero data from final time point data (dRFU).
3. Calculate the mean dRFU of the blank.
4. Subtract the mean blank dRFU from the dRFU of standards and samples (net dRFU).
5. Plot the standard curve, net dRFU vs. EU/mL (logarithmic axes). Use the individual net dRFU of each replicate of each CSE concentration in the range 0.005-5 EU/mL.
6. Calculate curve function by fitting a linear model to the data:
   log(net dRFU) = A * log(EU/mL) + B

Standard curve example

Note: Values may differ depending on the instrument gain.



8.2. Calculation of Endotoxin Concentration and Derived Data

1. Calculate the correlation coefficient |r|. It must be ≥ 0.980.
2. Calculate endotoxin concentration (EU/mL) of samples from net dRFU or dRFU using the functions mentioned above.
3. Average results of several replicates, multiply results with the dilution factors of the samples and compare to the samples' respective endotoxin limits.
4. Calculate the coefficient of variation (relative standard deviation) by dividing the standard deviation by the average of the replicates' endotoxin concentration. The recommended limit is of 25%.
5. Calculate the endotoxin concentration difference between – if necessary, diluted – sample and respective Positive Product Control. Determine if it is within 50% to 200% of the expected value, such as 0.25 to 1 EU/mL for a spike of 0.5 EU/mL.

9. Quality Control

This assay has been designed and developed to meet the most stringent quality requirements. The results of quality control are given on the quality control certificate available from our website (www.biomerieux.com).

10. Influencing Parameters and Limitations

Test interference can cause invalid results, as revealed by invalid spike recovery due to alteration of the enzymatic reaction conditions. Usually, such interference can be overcome by sample dilution in endotoxin-free water. The maximum valid dilution factor (MVD) is calculated using the formula below, where endotoxin limit is the maximum acceptable endotoxin concentration in the undiluted sample and assay sensitivity is the lowest standard concentration, such as 0.005 EU/mL for this assay:

$$MVD = \frac{\text{Endotoxin limit}}{\text{Assay Sensitivity}}$$

If interference of a sample cannot be overcome by valid dilution or other sample treatment, this test method is not suitable. In those cases, we recommend using the ENDOLISA® Endotoxin Detection Assay.

Another phenomenon distorting endotoxin testing is endotoxin masking or Low Endotoxin Recovery (LER).

LPS are amphiphilic and tend to aggregate in aqueous solutions. Under certain circumstances, the aggregation state of LPS can convert from a highly Factor C-activating state to a less or non-activating state. In case of LER, sample ingredients demonstrate such a direct impact on the structure of endotoxin. In contrast to test interference, LER is time-dependent and dilution-independent. For samples showing LER, the ENDO-RS® Endotoxin Recovery Kit can demask endotoxin which can be detected with the ENDOLISA® assay, i.e. to allow for valid time-independent endotoxin recovery.

Potential Test-Influencing Parameters

- The optimum temperature to perform the assay is +37°C.

- Samples with extreme pH values may influence assay performance, if the buffer capacity of the test system is exhausted. Dilution or pH adjustment to pH 7 is recommended.
- Total salt concentration in a sample should not exceed 500 mM. Otherwise, dilution is recommended.
- Detergents may interfere with this assay. Dilution is recommended. By forming mixed aggregates with endotoxin, they may stimulate LER.
- Chelating agents (such as Ethylene diamine tetraacetic acid (EDTA), Ethylene glycol tetraacetic acid (EGTA) and citrate) absorb cations. If such agents are present, dilution or neutralization of the chelating agent, for example with magnesium, is recommended. By destabilizing endotoxin, chelating agents may stimulate LER.
- Chaotropic agents may modulate hydrophobic interactions and denature Factor C. Dilution is recommended. ENDOLISA® is very robust against chaotropic agents.
- Organic solvents may modulate hydrophobic interactions and denature Factor C. Dilution is recommended. ENDOLISA® is very robust against organic solvents.
- Protein interference strongly depends on the physical and chemical properties of the proteins. Dilution is recommended. ENDOLISA® is very robust against protein interference. By absorbing endotoxin, proteins may stimulate LER.
- Serine proteases/peptidases like trypsin may mimic Factor C's activity and cause false-positive results, i.e. fluorescence development in the absence of endotoxin. Proteases can be revealed by recording the reaction kinetics of this assay, i.e. by reading fluorescence several times throughout the assay runtime. The reaction curve of Factor C is parabolic, while other proteases usually show linear reaction kinetics. In this case, heat treatment at +75°C for 15 minutes is recommended. Alternatively, protease inhibitors can be used in combination with ENDOLISA® (avoid EDTA).
- This assay is generally not suitable for the direct detection of endotoxin in serum, plasma or blood samples.

11. 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.

12. Troubleshooting

Observation	Possible Cause	Measure
No signal at all	• Inappropriate instrument settings	⇒ Check instrument parameter
	• Lamp defect	⇒ Change lamp
	• Pipetting error	⇒ Check reagents, repeat assay
	• Incubation temperature much too high or much too low	⇒ Check temperature setting
No signal with individual samples	• Pipetting error (no water or sample pipetted)	⇒ Repeat assay
	• Interfering ingredients	⇒ Check spike control; dilute sample 1:10
	• Inappropriate pH	⇒ Check pH, neutralize sample

Observation	Possible Cause	Measure
Low signal level	• Instrument sensitivity (gain) too low	⇒ Enhance sensitivity; higher gain needed
	• Reader defect (such as optics)	⇒ Run instrument check
	• Incubation temperature too high/too low	⇒ Check temperature
	• 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 should be measured around 445 nm; band 20-40 nm
High background signal in standards and negative control	• LPS contamination of assay components (such as 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 should not be below 360 nm, band 10-20 nm
High well-to-well variation	• Temperature gradient (incubator, reader)	⇒ Change incubator, reader
	• Pipetting error	⇒ Calibrate pipettes
	• Introduction of fluorescence-increasing material from the environment	⇒ Keep the tubes and strips covered by caps and scaffold lid as much as possible. Keep laboratory and reader clean. Consider using a laminar flow hood cabinet with disabled air flow.
Invalid spike control	• Interfering ingredients	⇒ Dilute sample
	• Inappropriate pH	⇒ Check pH; neutralize sample

13. Validation and Regulatory Information

Recombinant Factor C is included in the European Pharmacopeia (Ph. Eur.) Chapter 2.6.32 "Test for bacterial endotoxins using recombinant factor C".

In the FDA Guidance for Industry on Pyrogen and Endotoxins Testing: Questions and Answers, Recombinant Horseshoe Crab Factor C is included as an alternative method in Section 5.

Additional Guidelines for validation of alternative methods can be found in the United States Pharmacopoeia (USP) chapter 1225 and Ph. Eur. chapter 5.1.10.

General information on performing bacterial endotoxin testing can be found in the harmonized chapters of the USP chapter 85, Ph. Eur. chapter 2.6.14 and Japanese Pharmacopoeias (JP) chapter 4.01.

14. Index of Symbols

Symbol	Meaning
	Catalogue number
	Manufacturer
	Date of manufacture

Symbol	Meaning
	Contains sufficient for <n> tests
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Do not re-use

15. Limited Warranty

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, 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.

16. 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

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2021-06	058632-01	N/A	Not applicable (First publication)
2023-06	058632-02	Technical change	Explanation and Principle / Warnings and Precautions / Procedure / Calculation of Results / Troubleshooting

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