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Endotoxin detection – which method is best for me?

Endotoxin detection methods

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LAL Assays

- Gel clot
- Semi-quantitative gel clot
- *Endpoint chromogenic*
- *Endpoint turbidimetric*
- Kinetic chromogenic
- Kinetic turbidimetric

Recombinant Factor C Assays

- Endpoint fluorescent

Which test is best?

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Each test has its own place

The choice of test for a laboratory depends on:

- Result required
  - Quantitative/Semi-quantitative
  - Qualitative
- Available equipment
- Sample to be tested

Lonza PYROGENT® Gel Clot LAL Assay

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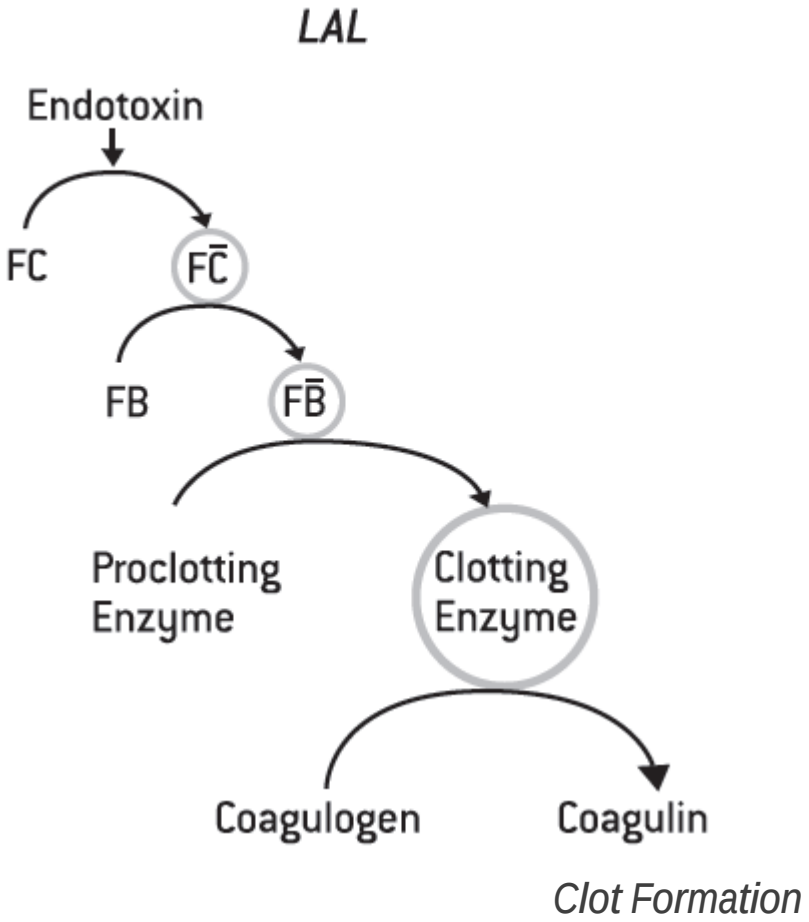
- PYROGENT® Kits
  - lysate only (matched, lyophilized control standard endotoxin (CSE) can be purchased separately)
- PYROGENT® Plus Kits
  - lysate and matched, lyophilized CSE
- Multiple sensitivities and vial sizes available

Vial size	Available sensitivities (EU/mL)
50 tests/vial	0.03, 0.06, 0.125, 0.25
16 tests/vial	0.06, 0.125

- Multiple kit configurations – standard sizes and bulk

Gel clot assay



Performing the gel clot assay

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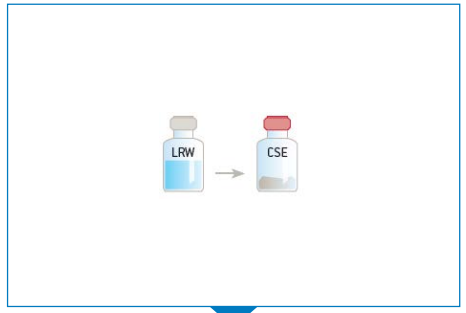
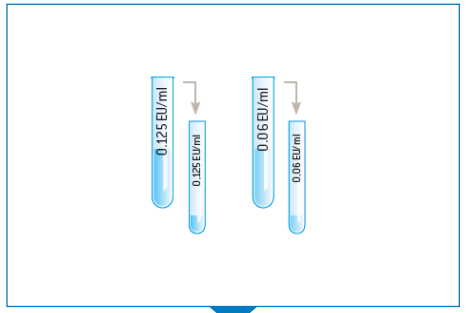
Step 1 → Step 2 → Step 3 → Step 4 → Step 5

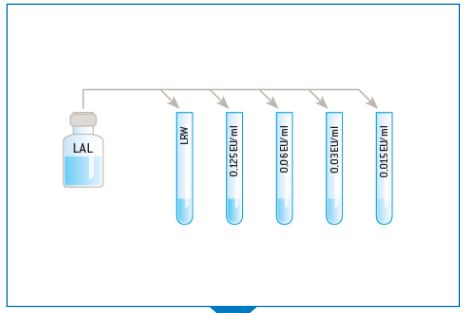


Reconstitute CSE as per package insert

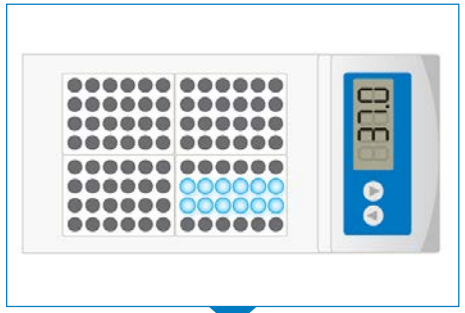


Prepare standards, samples and controls and pipette into reaction tubes

Reconstitute lysate as per package insert



Add lysate to reaction tubes



Incubate at 37°C for 60 minutes (+/- 2 minutes)

Reading the gel clot assay

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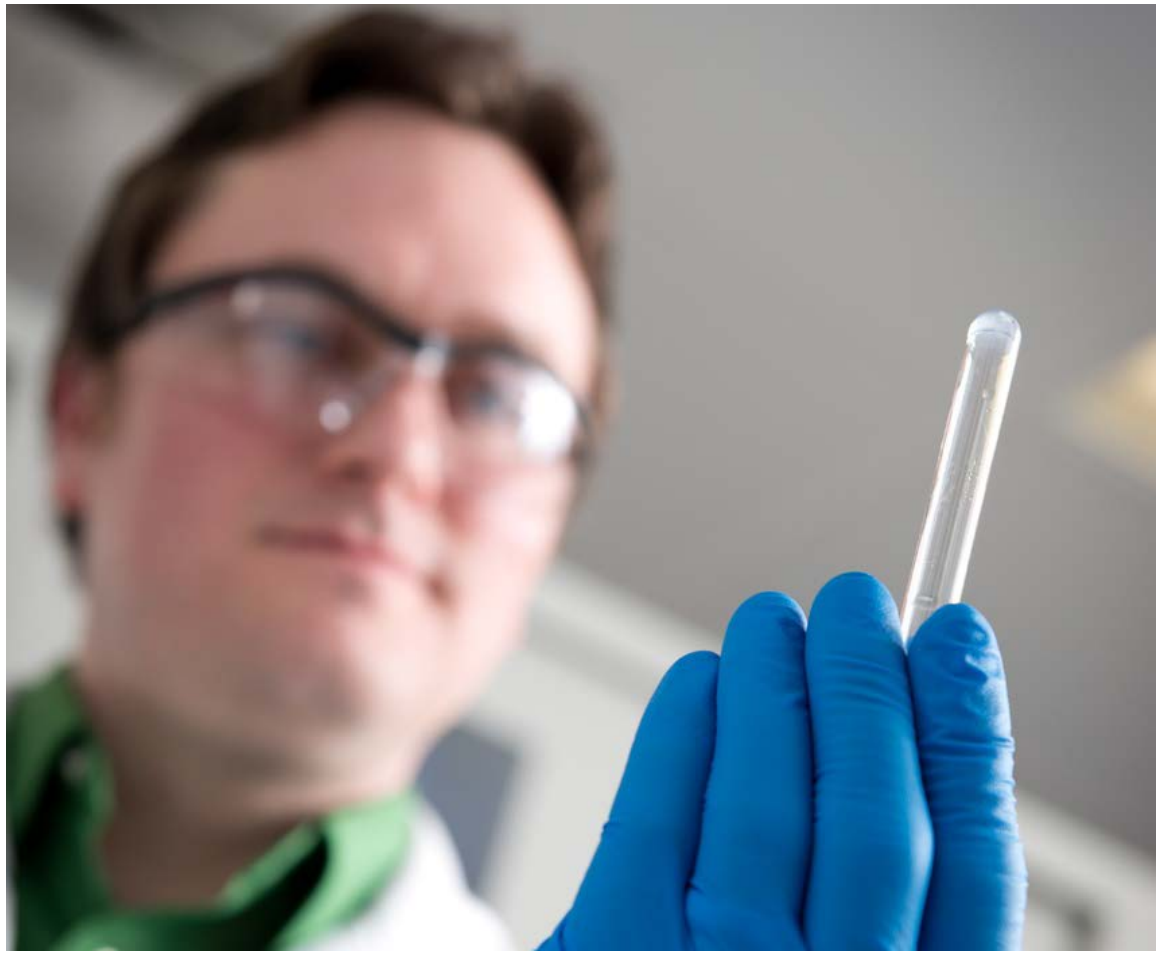
- Invert tube 180° in a smooth motion
- A positive reaction is characterized by the formation of a firm gel that remains intact
- A negative reaction is characterized by the absence of a solid gel after inversion



PYROGENT® Gel Clot LAL Assay

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Advantages

- A simple test to perform
- Low start-up costs
- No readers/software necessary

Disadvantages

- Results are subjective
- Gel formation is prone to interference
- Labor intensive
- Qualitative
  Semi-quantitative with many dilutions

Gel clot assays

The gel clot assay can be performed in two ways:

- Limit test
- Semi-quantitative

Gel clot assays

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Limit Test

- Yes/No answer to a specific kit sensitivity (more than/less than labeled lysate sensitivity)
- Positive control, negative control, sample and sample positive product control (PPC)

Semi-quantitative

- Standard series of endotoxin
- Dilution series of sample
- PPC most concentrated sample
- Negative control
- Sample endotoxin load range = Dilution factor of last positive (clotting) sample x kit sensitivity

Kinetic LAL assays

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01

The most recently developed LAL assays

02

Most objective of all the LAL assays

03

Two forms of kinetic LAL assays are available:

- Kinetic turbidimetric
- Kinetic chromogenic

Lonza kinetic LAL assays

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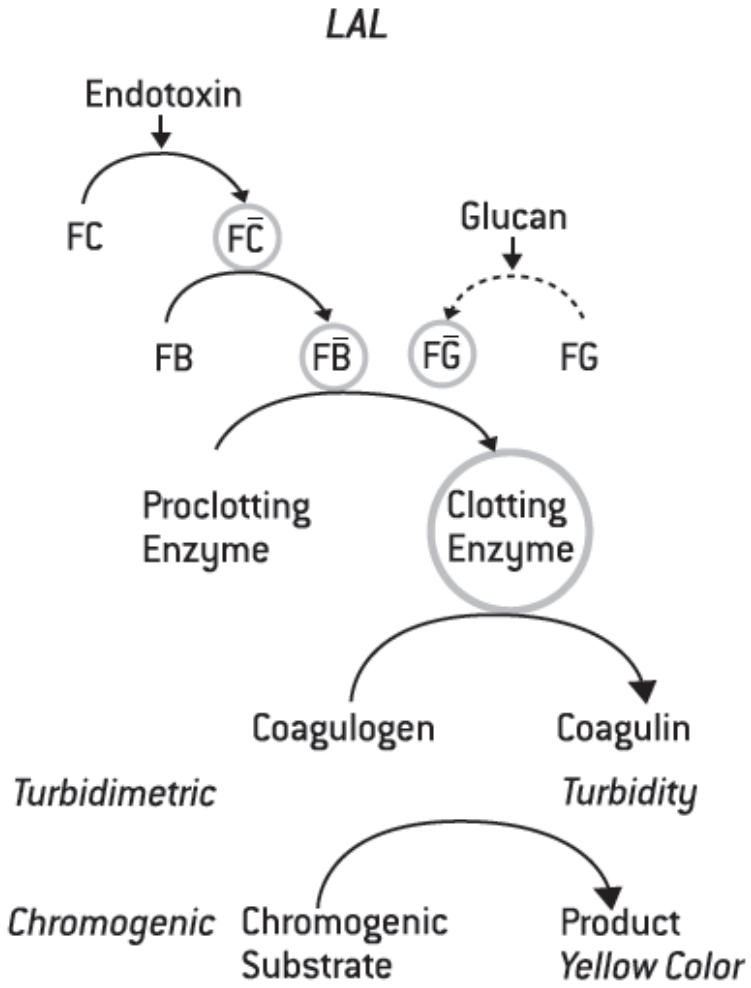
- Kinetic turbidimetric – Lonza PYROGENT®-5000 Assay
- Kinetic chromogenic – Lonza Kinetic-QCL® Assay
- Both kinetic LAL assay types are quantitative and can be conducted using an eight-channel incubating plate reader (ELx808™ Reader) with supporting software (Lonza WinKQCL® Software), provided the correct filters are used:
  - PYROGENT®-5000 Assay – 340 nm filter
  - Kinetic-QCL® Assay – 405 nm filter

Kinetic assays



Performing the kinetic assay

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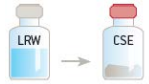
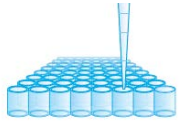
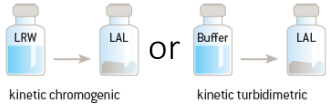
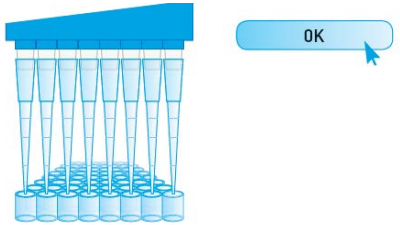
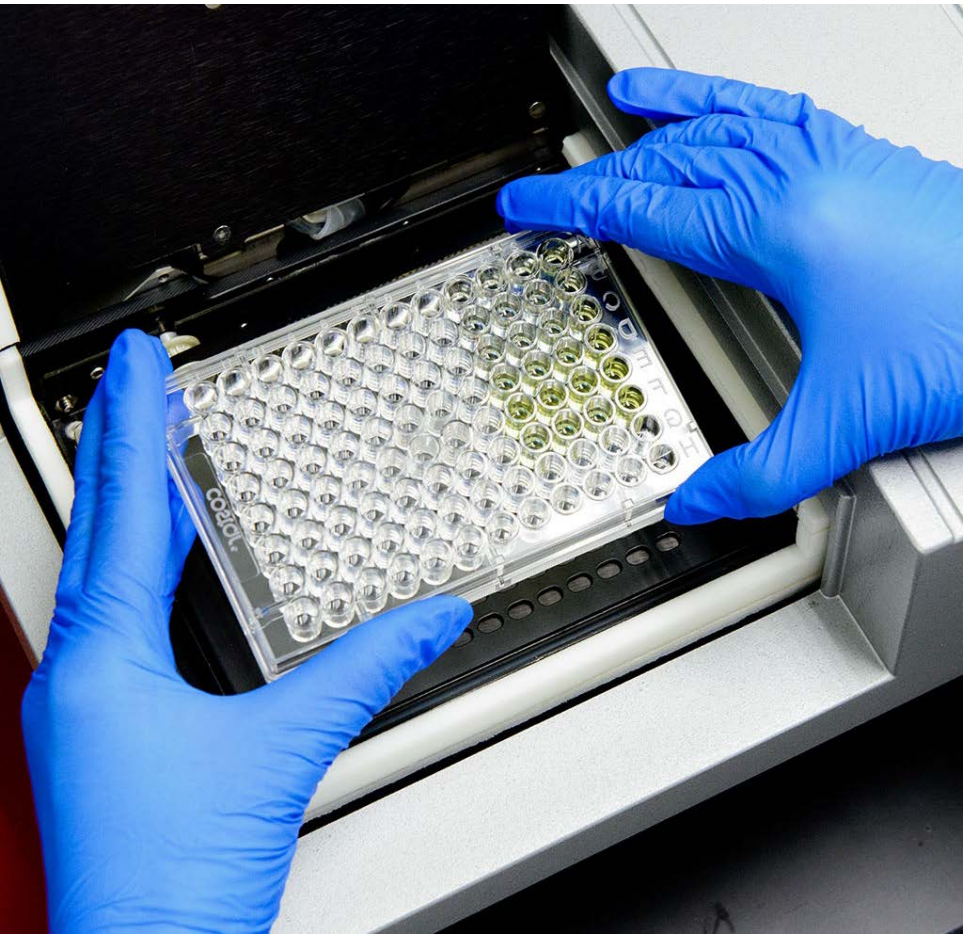
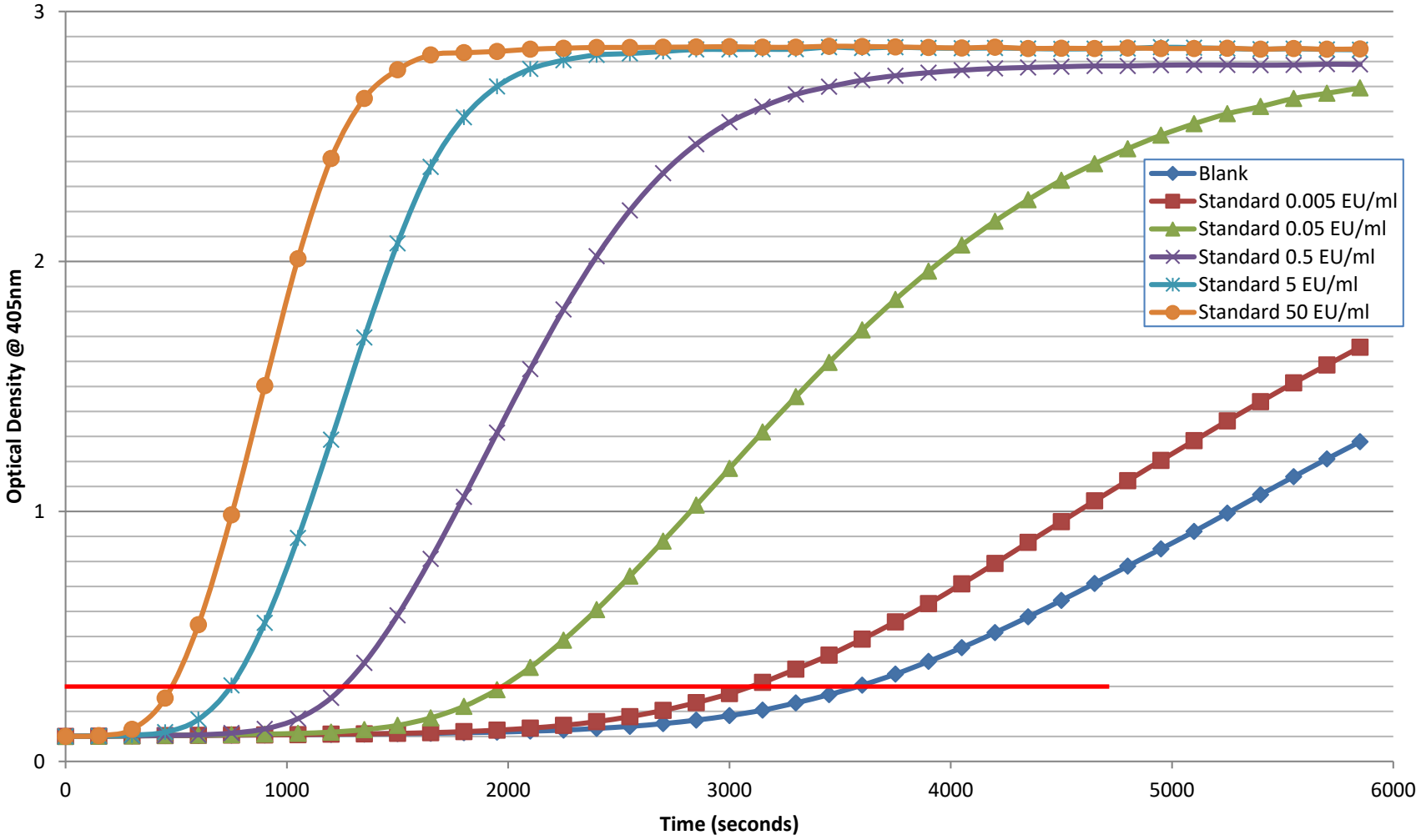
Step 1	Step 2	Step 3	Step 4	Step 5
				
Reconstitute CSE as per package insert	Prepare standards, samples and controls and pipette into 96-well plate	Pre-incubate the plate for 10 minutes at 37°C	Near the end of the pre-incubation period, reconstitute lysate as per package insert	Add lysate to 96-well plate and start the assay

How kinetic assays work



- The optical density in each well is read at the start of the assay
- All results from the first reading are set to a baseline level to account for color, opacity and more
- The reader measures the optical density at fixed time intervals looking for a delta mOD of 30 for the kinetic turbidimetric assay and a delta mOD of 200 for the kinetic chromogenic assay
- The ‘end of assay’ mark is reached when the lowest standard has reached the required mOD change
- The time taken for each well to reach the required mOD change is known as the reaction time

Kinetic LAL reaction



The kinetic assay

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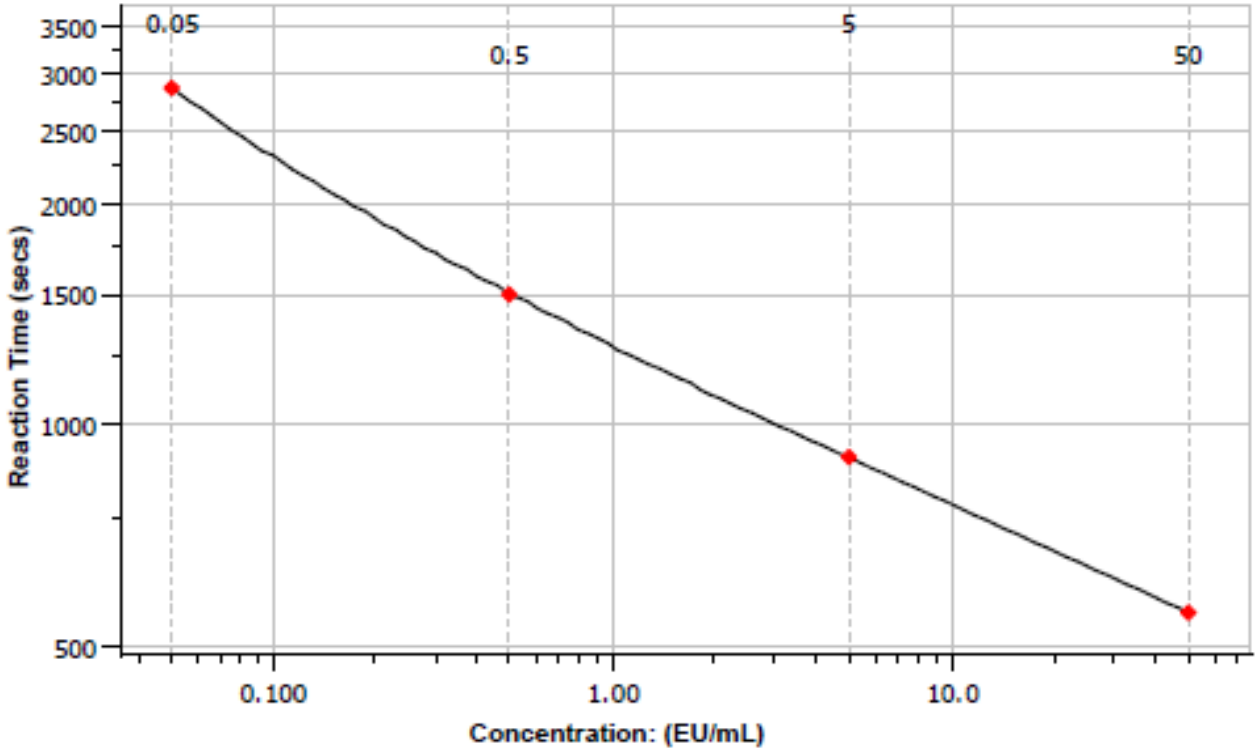
The mean reaction time for each of the endotoxin standards is plotted against endotoxin content using linear regression or PowerCurve™ to create a standard curve

The non-linear nature of the data means that at a minimum, the data must be subject to a log-log transformation to plot the standard curve

Mean reaction times for the sample and sample PPCs are used to calculate interpolated endotoxin content from this standard curve and reported in the relevant units – EU/ml or EU/mg – according to the endotoxin limit entered into the product details

Reaction time versus endotoxin concentration



A standard curve for the kinetic turbidimetric assay.

Note the log-log ratio of the standards and also the curve slopes downward – as more endotoxin equals a faster assay reaction time.

PYROGENT®-5000 Kinetic Turbidimetric LAL Assay

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Advantages

- Quantitative over a large range – 0.01 to 100 EU/ml
- Good for testing water and simple products
- Lower reagent cost than kinetic chromogenic assay
- Can cope with yellow-colored products which absorb in the 405 nm range (i.e. unsuitable for chromogenic testing)

Disadvantages

- Less sensitive than the Kinetic-QCL® Assay
- Similar interference problems as in the gel clot method and not suitable for turbid or viscous products
- Prone to problems from bubbles
- Requires a reader, computer and software

Kinetic-QCL® Kinetic Chromogenic LAL Assay

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Advantages

- Less subject to interference and copes with turbid or more viscous products
- Lonza's most sensitive LAL assay
- Quantitative over a large range – 0.005 to 50 EU/ml

Disadvantages

- Requires a reader, computer and software
- Not suitable for colored products which absorb in the 405 nm range

A sustainable alternative to LAL – PyroGene® Assay

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- PyroGene® Recombinant Factor C Assay is an animal-free, sustainable alternative to LAL-based assays
- This assay has several major advantages over conventional LAL assays
- The future of endotoxin testing

Recombinant factor C endotoxin detection assay

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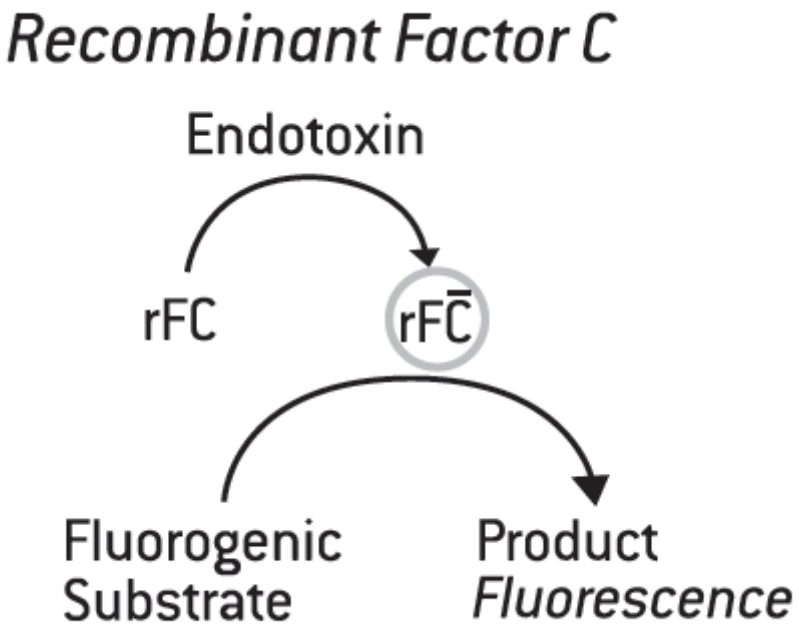

PyroGene® Assay overview

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- Single step quantitative endpoint fluorescent assay
- Assay takes one hour at 37°C
- Uses liquid reagents
- Performed in standard 96-well microplates
- The PyroWave® XM Reader is controlled by WinKQCL® Software

PyroGene® Assay features

- Consistent assay performance
- Security of supply
  - No animal utilization
- Specificity for endotoxin
  - Elimination of glucan reaction pathway removing false positive issue seen with LAL
- Comparability to LAL-based methods in terms of performance and pricing

Performing the PyroGene® Assay

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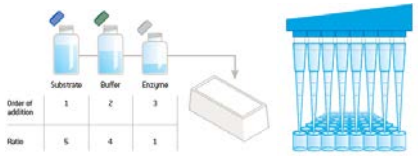
Step 1	Step 2	Step 3	Step 4	Step 5
				
Reconstitute CSE as per package insert and prepare a 4-point standard curve 0.005 to 5 EU/ml	Prepare standards, samples and controls and pipette into 96-well plate	Pre-incubate the plate for 10 minutes at 37°C	Prepare working reagent according to package insert (ratio 5:4:1, substrate:buffer:enzyme) and add to each well	Start assay

Reading the PyroGene® Assay

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01

WinKQCL® Software will take the first read for all wells and store them

02

The plate is then incubated for 60 minutes at 37°C

03

At the end of the assay the plate is read again, and the readings stored

PyroGene® Assay – calculations

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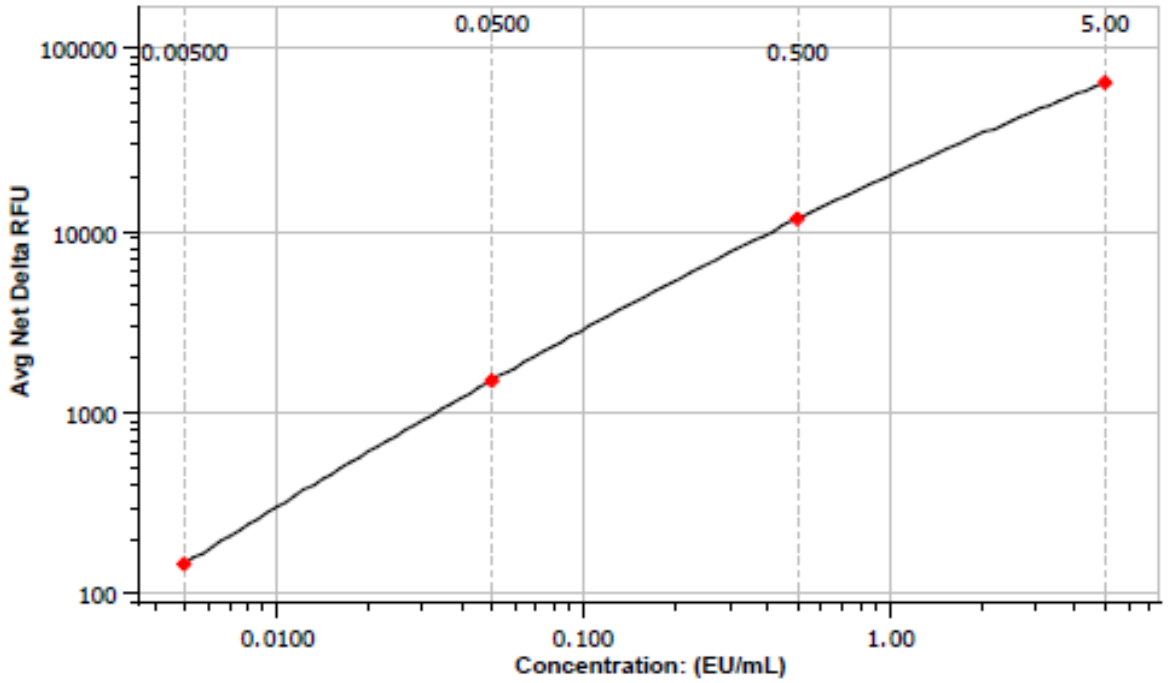
WinKQCL® Software then calculates:

- ΔRFU = RFU60 min minus RFUtime 0
- Averages ΔRFU for BLANKs
- Subtracts mean ΔRFUblank from all ΔRFUs

WinKQCL® Software then constructs a log-log plot of ΔRFU versus endotoxin concentration

Unknowns (samples and sample PPCs) are determined using this standard curve

PyroGene® rFC Assay standard curve



A standard curve for the PyroGene® rFC Assay.

The standard curve has a low RFU for the 0.005 EU/ml standard and a high RFU for the 5 EU/ml standard.

Endotoxin assay overview

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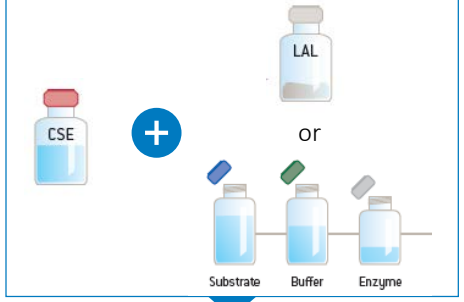
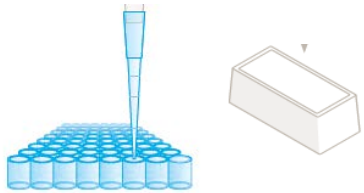
Sample → Endotoxin Assay → Consumables → Reader → WinKQCL® Software



- PYROGENT®-5000 Kinetic Turbidimetric LAL Assay
- Kinetic-QCL® Kinetic Chromogenic LAL Assay
- PyroGene® Recombinant Factor C Assay



- 96-well plates
- Pipette tips
- Reagent reservoirs
- Pyrogen-free dilution tubes

or

- Fully integrated solution for endotoxin detection and analysis
- Built with components to meet the latest data integrity requirements

Summary of endotoxin detection methods

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- LAL Assays
  - Gel clot
  - Semi-quantitative gel clot
  - *Endpoint chromogenic*
  - *Endpoint turbidimetric*
  - Kinetic chromogenic
  - Kinetic turbidimetric
- Recombinant Factor C Assays
  - Endpoint fluorescent

Choice of assay is dependent on…

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	Gel clot	Kinetic turbidimetric	Kinetic chromogenic	rFC assay
Workload	● ● ●	●	●	●
Nature of the samples to be tested	Water, in-process and final release testing. Also suitable for plant-based material and acidic/basic samples	Water samples, simple products and yellow-colored products which absorb in the 405 nm range	Biological products (i.e. vaccines and antibiotics) and turbid or viscous samples	Water, in-process, final release testing. Also suitable for plant-based material
Available equipment or equipment budget	Dry heat block or water bath	Incubating absorbance reader	Incubating absorbance reader	Incubating fluorescence reader
Need for quantitation	Qualitative (Yes/No answer)	Quantitative (Results calculated from a standard curve)	Quantitative (Results calculated from a standard curve)	Quantitative (Results calculated from a standard curve)

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Thank You