KINETIC ENDOTOXIN DETECTION SYSTEMS

acc
PROTECTION
THROUGH
DETECTION

It's Official!

The US Pharmacopeia (USP) Chapter <86>, "Bacterial Endotoxins Test Using Recombinant Reagents," will allow the use of non-animal-derived reagents for endotoxin testing — which includes **PyroSmart NextGen®**.

First-Gen. **Second-Gen. NEXT-GEN.**

Wherever you are on your BET journey, we've got you covered.

BETransformed. ACC transformed endotoxin testing in 1974 with the introduction of its Pyrotell® lysate gel-clot reagent and then again with its chromogenic and turbidimetric tests, Pyrochrome® and Pyrotell®-T.

Now, we are transforming the industry again with **PyroSmart NextGen®**, a groundbreaking recombinant BET solution with all of the quality and consistency you have come to expect from our traditional LAL reagents.

As you navigate your own transformation journey — from qualitative to quantitative to recombinant — count on ACC for the highest-quality products and support.

Learn more at **acciusa.com/BETransformed**.

PyroSmart®
NEXTGEN Recombinant Cascade Reagent (rCR)

Kinetic Chromogenic Method Recombinant Reagent

PyroSmart®
NEXTGEN Recombinant Cascade Reagent (rCR)

PyroSmart NextGen® recombinant cascade reagent (rCR) marks the introduction of a new sustainable recombinant LAL reagent technology for bacterial endotoxin testing (BET). Utilizing the same LAL cascade as traditional LAL reagents while eliminating the potential for (1→3)-β-D-glucan cross-reactivity, PyroSmart NextGen® delivers all of the quality and consistency of results you have come to expect from ACC LAL reagents.

The US Pharmacopeia (USP) Chapter <86> "Bacterial Endotoxins Test Using Recombinant Reagents" will allow the use of non-animal-derived reagents for endotoxin testing. With this approval, we can help our customers transition from naturally sourced BET reagents to PyroSmart NextGen®, a shift that will strengthen the supply chain and enhance sustainability.

PyroSmart NextGen® can be used for a wide variety of endotoxin tests, ranging from standard water testing to samples requiring high sensitivity, such as intrathecal products and those requiring high dilutions to overcome interference.

Sensitivity

The sensitivity for recombinant chromogenic assays is determined by the lowest standard concentration on the standard curve used for the assay. The maximum sensitivity of PyroSmart NextGen® is 0.005 EU/mL when run in an incubating microplate reader (or 0.001 EU/mL when run in Pyros Kinetix® Flex tube reader).

Sample to Lysate Ratio

PyroSmart NextGen® is used with an economical volume of 50 µL of reagent per well, yielding 50 tests/vial:

- *Microplate reader:*
  1:1 ratio using 50 µL of test sample : 50 µL of reagent

Performing the Test

The PyroSmart NextGen® reaction mixture is incubated at 37±1°C and read in a microplate reader equipped with a 405–410 nm filter. The time of incubation is dependent on the lowest standard concentration in the standard curve, with 0.005 EU/mL achievable in 2,500 seconds in a microplate reader. Software is used to construct the standard curve and calculate the endotoxin concentrations.

Reconstitution

PyroSmart NextGen® is provided as co-lyophilized with the chromogenic substrate. As such, it is ready to use following a simple reconstitution (using 2.8 mL of the supplied reconstitution buffer).

Stability

PyroSmart NextGen® is a lyophilized product with an excellent shelf life of 3 years from the date of manufacture.

Packaging

PyroSmart NextGen® reagent is provided as a pack of 2 vials of reagent and 2 vials of reconstitution buffer. This is sufficient for a total of 110 wells (55 wells per vial).

Kinetic Chromogenic Method Recombinant Reagent

Continued

Keep Your Method. Make an Impact.

PyroSmart NextGen® is a sustainable recombinant cascade reagent (rCR) that delivers the same reliable results as your conventional LAL reagent and offers these additional advantages:

- No animal content — horseshoe-crab-blood-free
- Same cascade
- No cross-reactivity with (1→3)-β-D-glucan
- Same instrument
- Same preparation steps
- Meets your sustainability objectives
- Approved methodology under USP Chapter <86>, "Bacterial Endotoxins Test Using Recombinant Reagents"

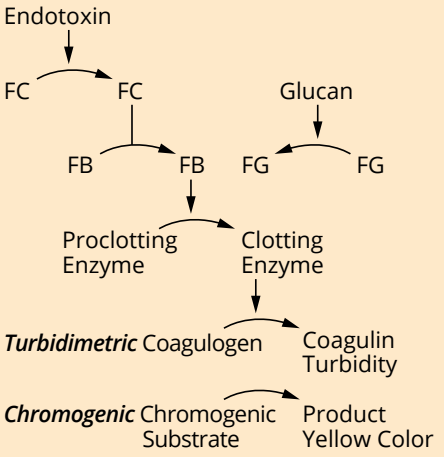
ACC's PyroSmart NextGen® uses the same cascade as traditional LAL reagents by manufacturing the factors responsible for the cascade using recombinant processes. As a result, our new recombinant reagent's mechanism of action will deliver results consistent with traditional LAL reagents. It offers the added advantage of eliminating (1→3)-β-D-glucan cross-reactivity from the LAL cascade, since there is no Factor G in the final reagent. ACC developed PyroSmart NextGen® to provide a sustainable alternative to traditional, naturally sourced LAL reagents, while allowing customers to maintain their lab procedures, methods, instrumentation, and, most importantly, their results.

LAL Reagent Comparison — The Benefits Are Clear

	Traditional LAL Reagent	ACC's PyroSmart NextGen® Recombinant Cascade Reagent (rCR)	Competitor Recombinant Factor C Reagent (rFC)
Year Technology Introduced	1977	**2021**	2003
Kinetic Assay	✓ Yes — Kinetic	✓ **Yes — Kinetic**	✗ No — Endpoint only
Assay Setup	✓ Single Step Reconstitution	✓ **Single Step Reconstitution**	✗ No — rFC requires three reagents in a 1:4:5 ratio and a 10-minute pre-incubation step
Same Standard Plate Reader	✓ Yes — Incubating plate or tube reader at 405 nm	✓ **Yes — Incubating plate or tube reader at 405 nm**	✗ No — Fluorescent reader required
Derived From Limulus Amebocyte Lysate (LAL)	✓ Yes — LAL	✓ **Yes — rCR is recombinant LAL**	✗ No — Based on Carcinoscorpius or Tachypleus Amebocyte Lysate (CAL or TAL)
Multi-step Cascade Pathway	✓ Yes	✓ **Yes**	✗ No
Endotoxin Specific	✗ No	✓ **Yes**	✓ Yes
Sustainable Reagent (animal free)	✗ No	✓ **Yes — Horseshoe-crab-blood-free**	✓ Yes — Horseshoe-crab-blood-free

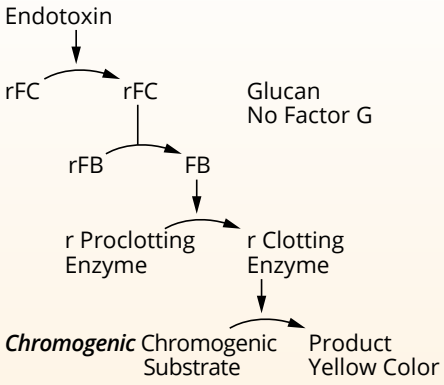
The Importance of Mechanism of Action Recombinant Cascade Reagent (rCR)

TRADITIONAL LAL REAGENT



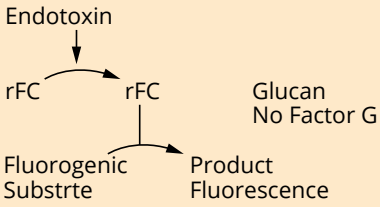
In the presence of endotoxin, Factor C becomes an activated moiety that in turn activates Factor B and the proclotting enzyme, ultimately resulting in the proteolytic cleavage of a substrate (either coagulogen in gel clot and turbidimetric assays or a colorless chromogenic substrate in chromogenic assays). The cascade mechanism thus amplifies the response of Factor C and leads to an exceptional sensitivity for this biological assay, with kinetic output being preferable. In the presence of (1→3)-β-D-glucan, Factor G becomes an activated moiety that also activates the proclotting enzyme, resulting in the same signal as that triggered by endotoxins through Factor C. This has been often observed as glucan-derived enhancement or false positive results.

RECOMBINANT CASCADE REAGENT (rCR)



As with naturally sourced LAL reagents, in the presence of endotoxin, recombinant Factor C becomes an activated moiety that in turn activates recombinant Factor B and the recombinant proclotting enzyme, ultimately resulting in the proteolytic cleavage of a colorless chromogenic substrate formulated with PyroSmart NextGen®. By relying on the same cascade mechanism, the response of recombinant Factor C is amplified the same way as by LAL reagents and thus the same sensitivity is achieved using this kinetic assay. Due to absence of Factor G, PyroSmart NextGen® will not react with any (1→3)-β-D-glucan and therefore will prevent glucan-derived enhancement and false positive results.

RECOMBINANT FACTOR C REAGENT (rFC)



Launched almost two decades ago, rFC reagents rely only on a recombinant form of Factor C. Due to the absence of the cascade as the amplification mechanism, rFC reagents are paired with a fluorescence method instead. However, this constitutes a different measured entity, different instrumentation, and different preparation steps with a limited output (endpoint assay only). Therefore, the uptake and implementation of this method has been rather limited.

Converting to PyroSmart NextGen® Is Easy

Switching to this sustainable alternative is easy because PyroSmart NextGen® follows the same cascade pathway as traditional reagents.

Kinetic Chromogenic Method Reagents

Pyrochrome®

Kinetic Chromogenic LAL Reagent

Pyrochrome® can be used for a wide variety of sample types, ranging from standard water testing to samples requiring high sensitivity, such as intrathecal products and those requiring high dilutions to overcome interference.

The BET reagent is formulated with a synthetic substrate that produces a chromophore when cleaved by endotoxin-activated enzyme. The test is read in a tube reader or an incubating microplate reader.

Sensitivity

The maximum sensitivity of Pyrochrome® is 0.001 EU/mL when run in Pyros Kinetix® Flex tube reader or incubating microplate reader with Glucashield® Buffer.

Sample to BET Ratio

In the Pyros Kinetix® Flex tube reader, Pyrochrome® can be used at an economical ratio of 4:1 using 50 µL of reagent per well or at 1:1 using 100 µL/well.

In a microplate reader, the reagent is used at a ratio of 1:1 and a volume of 50 µL/well (60 tests/vial) or 100 µL/well (30 tests/vial).

Performing the Test

The Pyrochrome® sample mixture is incubated in an optical reader at 37±1˚C and read at a wavelength of 405 nm. No pre-incubation is required and results can be available within 1 hour. However, time to results is dependent on the required assay sensitivity. Software will analyze the data to provide endotoxin results.

Reconstitution

Pyrochrome® lysate is reconstituted with an optimized Pyrochrome® reconstitution buffer (C1500-5). Pyrochrome® can also be reconstituted with Glucashield® buffer (CG1500-5), a (1→3)-β-D-glucan-inhibiting buffer, to render the assay endotoxin specific.

Stability

Once reconstituted, Pyrochrome® is stable for 8 hours when stored at 2–8˚C.

Packaging

Pyrochrome® is offered with a choice of reconstitution buffer and is recommended for use with the 10 ng/vial Control Standard Endotoxin (CSE, EC010-5). Certificates of Analysis, specific to the Pyrochrome® and CSE lot, can be obtained from ACC or online at acciusa.com.

Chromo-LAL

Kinetic Chromogenic Formulation

Chromo-LAL is optimized for the kinetic chromogenic BET test method in microplate readers. Chromo-LAL is a buffered, stable and robust lysate, suitable for quantitative testing of a wide range of samples.

Sensitivity

The sensitivity for chromogenic assays is determined by the lowest standard concentration on the standard curve used for the assay. The maximum sensitivity of Chromo-LAL is 0.005 EU/mL.

Sample to BET Ratio

Reconstituted Chromo-LAL reagent is used at a ratio of 1:1 and a volume of 100 µL/well (30 tests/vial).

Performing the Test

The Chromo-LAL/sample mixture is incubated at 37±1°C and read in a microplate reader. Software is used to construct the standard curve and calculate the endotoxin concentrations.

Reconstitution

Chromo-LAL lysate is reconstituted with BET Reagent Water (LRW). It can also be reconstituted with Glucashield® buffer, a (1→3)-β-D-glucan-inhibiting buffer, to render the assay endotoxin specific.

Stability

Once reconstituted, Chromo-LAL is stable for 24 hours if stored at 2–8°C. Chromo-LAL may be frozen once and will retain activity for 2 weeks if stored at or below -20°C.

Packaging

Each vial contains reagent for approximately 30 tests. It is recommended for use with 0.5 µg/vial Control Standard Endotoxin (CSE, E0005-1). Certificates of Analysis, specific to the Chromo-LAL and CSE lot, can be obtained from ACC or online at acciusa.com.

Kinetic Turbidimetric Method Reagent

Pyrotell®-T

Kinetic Turbidimetric LAL Reagent

Pyrotell®-T turbidimetric reagent formulation is a versatile and cost-effective solution for the determination of endotoxin. The optical density (turbidity) increase that accompanies the clotting reaction is read in the Pyros Kinetix® Flex tube reader or in an incubating microplate reader.

When used with the Pyros Kinetix® Flex tube reader, Pyrotell®-T is a highly economic, flexible, and sensitive BET assay. It can be used for a wide variety of tests, ranging from water testing to samples requiring high sensitivity, such as intrathecal products and those requiring high dilutions to overcome interference.

Product Sensitivity

When used in a Pyros Kinetix® Flex tube reader, the maximum sensitivity is 0.001 EU/mL. The unique formulation of Pyrotell®-T allows a wide selection of standard curves to be used, giving the user flexibility, speed, and ease in performing assays.

Sample to BET Ratio

The ratio of sample to BET is determined by personal preference and sample chemistry (interference patterns). Reconstituted Pyrotell®-T reagent is used at a sample to lysate ratio of 1:1 or 4:1 and volume of 100 µL/well (48 tests/vial) or 50 µL/well (96 tests/vial), respectively.

Performing the Test

The Pyrotell®-T sample mixture is incubated in an optical reader at 37±1°C and read at a desired wavelength depending on the instrumentation and user choice. The time of incubation is dependent on the lowest standard concentration in the standard curve. Software is used to analyze the standard curve and calculate the endotoxin concentrations.

Reconstitution

Pyrotell®-T may be reconstituted with 5 mL of LAL Reagent Water (LRW), Pyrosol® buffer, or Glucashield® buffer, depending on the demands of the sample being tested. Pyrosol® buffer provides improved kinetics and extra pH buffering capacity. Glucashield® buffer, a (1→3)-β-D-glucan-inhibiting buffer, is used to render the assay endotoxin specific.

Stability

Once reconstituted, Pyrotell®-T is stable for 24 hours, if stored at 2–8°C. Pyrotell®-T may be frozen once and will retain activity for as long as 3 months if stored at or below -20°C.

Packaging

Pyrotell®-T is available in multi-test vials. Each vial contains reagent for approximately 96 tests (when used with the Pyros Kinetix® Flex tube reader and 4:1 sample to BET ratio) or 48 tests (when used with 1:1 ratio and/or in a microplate reader). It is recommended for use with the 0.5 µg/vial Control Standard Endotoxin (CSE, E0005-1). Certificates of Analysis, specific to the Pyrotell®-T and CSE lot, can be obtained from ACC or online at acciusa.com.

Kinetic Microplate System

For Efficient & Accurate Endotoxin Testing

Epoch 2 Microplate Spectrophotometer*

The BioTek® Epoch 2, manufactured by BioTek® Instruments (now part of Agilent Technologies), is an incubating absorbance microplate reader that, along with our Pyros® eXpress 21 CFR Part 11-compliant software, provides a complete system for efficient and accurate endotoxin testing.

It delivers excellent performance for UV-Vis absorbance measurements, which can be performed in 6- to 384-well microplates, cuvettes, and in microvolume samples. The broad wavelength range enables applications from nucleic acid and protein quantification in the low UV to microbial growth assays at higher wavelengths. A 4-Zone™ Temperature Control system and unique heated track/carrier design provide for minimal evaporation and edge effect.

System Specifications

- Filter-free UV-Vis wavelength selection from 200 to 999 nm in 1 nm increments
- Compatible with 6- to 384-well microplates and cuvettes for assay versatility
- Integration with ACC's Pyros® eXpress software
- Precise reporting
- Can be used for a wide range of applications

ACC recommends that customers confirm their expected product support window directly with Agilent Technologies.

*Trademark of BioTek Instruments, Inc.

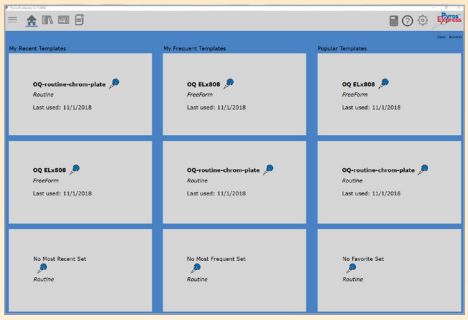
Analysis Software

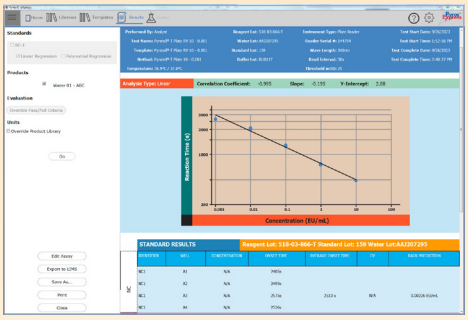
Pyros® eXpress

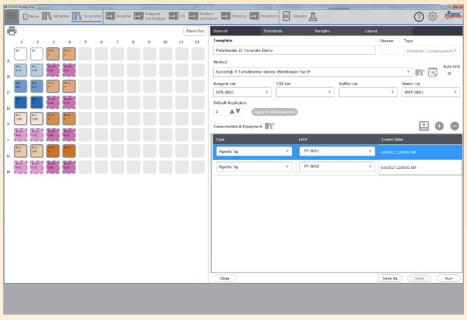
Endotoxin & Glucan Analysis Software

ACC introduces the next generation of endotoxin and glucan detection analysis software that offers integrated solutions for your quantitative endotoxin and glucan detection testing, reporting, trending, and data management needs.

Pyros® eXpress Software supports all of the quantitative endotoxin and glucan detection assays from ACC and allows users to quickly and efficiently test in a Quality Control environment. The Pyros® eXpress Software provides greater flexibility and versatility in the laboratory, allowing you to work smarter and faster while maintaining regulatory compliance. Pyros® eXpress Software meets 21CFR Part 11 technical requirements for electronic records, signatures, audit trails, as well as US and EU data integrity expectations.







EASE OF USE

- Custom templates available on the home screen provides quick-start options with minimal clicks to assay initiation.
- A fully integrated product validation workflow provides guidance for a systematic and compliant testing process.
- Custom permission settings help you control your testing environment and reduce laboratory errors:
  - » *Lysate/CSE matching to only allow the use of previously qualified reagents*
  - » *Optional technician qualification requirements*
  - » *Supply/equipment expiry safeguards*

EFFICIENCY

- Product-centric reporting with the industry plate and tube readers provides real-time results for individual samples.
- Reagent, product, and supply libraries help streamline test setup and reduce time spent on manual entry.

VERSATILITY

- Pyros® eXpress supports both plate and tube reader platforms, resulting in greater flexibility and laboratory throughput for endotoxin and glucan testing.

Kinetic System Ordering Information

PyroSmart NextGen® Multi-Test

2.8 mL/vial (approx. 50 tests/vial)

#PNG050-2	PyroSmart NextGen® with Reconstitution Buffer 2 pack (approx. 100 tests)

Pyrochrome® Test Kit

3.2 mL/vial (approx. 60 tests/vial)

#C1500-5	Pyrochrome® with Reconstitution Buffer 5 pack (300 tests)
#C1500-25	Pyrochrome® with Reconstitution Buffer 25 pack (1500 tests)
#CG1500-5	Pyrochrome® with Glucashield® Buffer 5 pack (300 tests)
#CG1500-25	Pyrochrome® with Glucashield® Buffer 25 pack (1500 tests)

Chromo-LAL

3.2 mL/vial (approx. 30 tests/vial)

#C0031-5	5 pack (150 tests)

Pyrotell®-T Test Kit

5 mL/vial (approx. 50 tests/vial)

#T0051-5	5 pack (250 tests)
#T0051-25	25 pack (1250 tests)

Control Standard Endotoxin

Escherichia coli O113:H10

#EC010-5	10 ng/vial (5 pack) for Pyrochrome®
#E0005-5	0.5 µg/vial (5 pack) for turbidimetric

Pyros Kinetix® Flex

Incubating Kinetic Tube Reader

#PKF96-PKG-D	Pyros Kinetix® Flex 96-well (includes instrument, Pyros® eXpress, validation doc & domestic power conditioner)
#PKF96-PKG-I	Pyros Kinetix® Flex 96-well (includes instrument, Pyros® eXpress, validation doc & international power conditioner)
#CAL07	On-site Calibration for Pyros Kinetix® Flex

Microplate Reader

#EPOCH2NS-SN-D	Epoch 2 Microplate Spectrophotometer (includes domestic power conditioner)
#EPOCH2NS-SN-I	Epoch 2 Microplate Spectrophotometer (includes international power conditioner)
#EPOCH2NS-SI-D	Epoch 2 Microplate Spectrophotometer (IVD) (includes domestic power conditioner)
#EPOCH2NS-SI-I	Epoch 2 Microplate Spectrophotometer (IVD) (includes international power conditioner)
#ELXP	Agilent/BioTek Universal Test Plate
#CALPR	On-site Preventative Maintenance and Performance Verification Service

Pyros® Express

Endotoxin and Glucan Analysis Software

#PEXS	Pyros® eXpress Software Package (USB media, 1 Workgroup License[1], 1 Reader License[2] and Software Support[3])
#PEXS-RL2	Pyros® eXpress Software Reader License
#PEXS-WL1	Pyros® eXpress Software Workgroup License
#PEXS-VAL-DOCS	Pyros® eXpress Software Validation Protocols (Pyros Kinetix® Flex and plate reader)
#PEXS-SUP3	Pyros® eXpress Software Annual Support
#PEXS-ADVS4	Pyros® eXpress Software Remote Advanced Support[4]
PEXS-VAL	Pyros® eXpress Software On-site Validation for Pyros Kinetix® Flex and plate reader
PEXS-OS	On-site Pyros® eXpress Software Support (plus travel expense)

1. Workgroup License: License allows software to be loaded on all computers connected to one Pyros® eXpress network database.
2. Reader License: Allows for one reader/instrument to be connected; standalone or as part of the network database.
3. Software Support can be provided through ACC's Field Service, Technical Services, and/or the Software Support Group and is provided for a period of 1 year from time of software purchase. Software support includes upgrades, patches, and basic assistance with software setup. This does not include advanced or on-site support; however, advanced and on-site support are available through our remote advanced and on-site support service offerings (refer to price list for options and pricing). Examples of basic support include, but are not limited to: assistance setting up products, accessories, and templates; running validation and endotoxin tests, trending data; software configuration of the Pyros® eXpress UI; and pre-installation and installation questions covered within the scope as defined in the software manual.
4. Advanced Support includes those support needs that are beyond scope of basic support as described above. It may require a fee for service (including travel costs). Please refer to our price list or speak with your Account Manager or Field Service Support Representative for pricing information. Examples of Advanced Support include, but are not limited to: database setup, maintenance, and troubleshooting; network, security, and firewall troubleshooting; report customization and import or export setup with external systems, such as environmental monitoring systems or laboratory information management systems.

acc™
PROTECTION THROUGH DETECTION

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