PyroSmart®

NEXTGEN Recombinant Cascade Reagent (rCR)

Product Brochure

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PROTECTION THROUGH DETECTION

The future of sustainable LAL is here! Associates of Cape Cod, Inc.'s (ACC) PyroSmart NextGen® recombinant Cascade Reagent (rCR) marks the introduction of the latest in sustainable recombinant LAL reagent technology for Bacterial Endotoxin Testing (BET). Unlike first generation recombinant reagents on the market, PyroSmart NextGen® uses the same LAL cascade as traditional LAL reagents, while eliminating the potential for 1,3-β-D-Glucan cross reactivity. PyroSmart NextGen® gives you the same complete cascade with all of the quality and consistency of results you have come to expect from ACC LAL reagents.

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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"

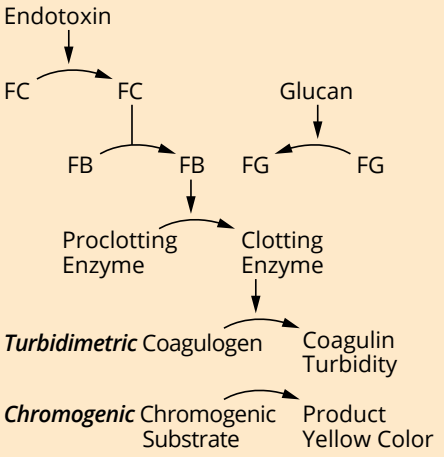
ACC's PyroSmart NextGen® uses the same cascade as traditional LAL reagents by manufacturing the factors responsible for the cascade using recombinant processes. As a result, our new recombinant reagent's mechanism of action will deliver results consistent with traditional LAL reagents. It offers the 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

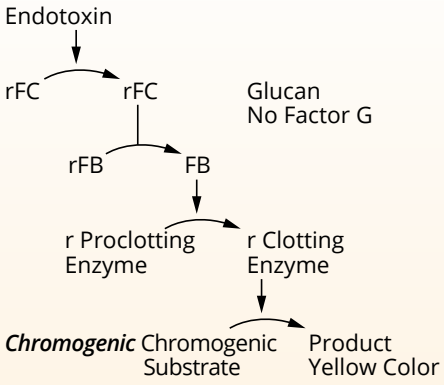
The Importance of Mechanism of Action Recombinant Cascade Reagent (rCR)

TRADITIONAL LAL REAGENT



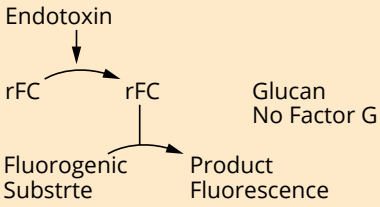
In the presence of endotoxin, Factor C becomes an activated moiety that in turn activates Factor B and the proclotting enzyme, ultimately resulting in the proteolytic cleavage of a substrate (either coagulogen in gel clot and turbidimetric assays or a colorless chromogenic substrate in chromogenic assays). 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.

PROTECTION THROUGH DETECTION

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