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Guide for Implementation of Recombinant Cascade Reagent (rCR) PyroSmart NextGen® for Bacterial Endotoxins Testing (BET) When Testing Per USP In Biopharmaceutical Manufacturing.

PyroSmart®
NEXT GEN Recombinant Cascade Reagent (rCR)

In an era where secure supply chain and sustainability are paramount, the implementation of PyroSmart NextGen® (PSNG) - recombinant Cascade Reagent (rCR) for endotoxin testing is a necessary step forward. Designed for QC managers, QC directors, Regulatory Affairs professionals, and Sustainability Officers, this guide provides a roadmap for adopting rCR in your quality control systems.

Disclaimer: The information provided in this document is given for the purposes of education and discussion. It is not intended to be, and it should not be used as, a substitute for regulation and regulatory guidance. Decisions and actions should be based on the relevant regulations, guidance documents and pharmacopeial chapters, not on this document. This guide provides information about implementation of PSNG reagent only which is the only widely published recombinant cascade reagent commercially available. The analytical performance of other recombinant cascade reagents should not be assumed to be identical to PSNG.

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Why Implement PyroSmart NextGen® Now?

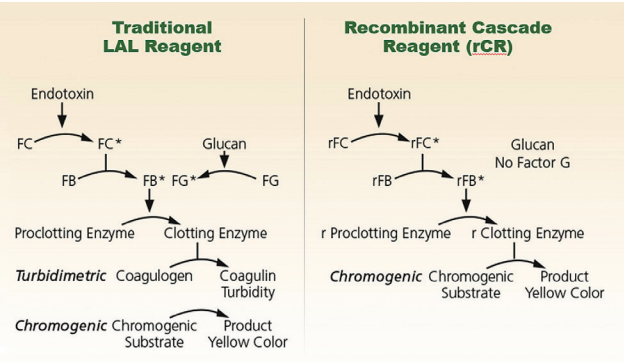
Implementing recombinant cascade reagent PyroSmart NextGen® (PSNG) offers several compelling benefits:

- Regulatory acceptance:
    1. *US Pharmacopeia Chapter <86> Bacterial Endotoxin Test Using Recombinant Reagents* (official as of 01 MAY 2025) includes recombinant cascade reagents (such as PSNG) as one of the options of recombinant reagents.[1]
    2. FDA Guidance for Industry 2026 Level 2 document "Pyrogen and Endotoxins Testing: Questions and Answers"[2] (released on 18 MAR 2026) as part of FDA's 2025 Roadmap to reducing animal safety testing in preclinical studies[3] provides flexibility for manufacturers to transition from the LAL reagents to recombinant reagents. On 20 APR 2026, the FDA released a follow up report summarizing the progress in implementing the roadmap and clarifying the next steps.[4] "Available data shows that the recombinant reagents deliver equivalent performance without the harvesting and use of marine animals. The transition to use of recombinant reagents for bacterial endotoxin testing also demonstrates how alternatives can address multiple concerns simultaneously—animal welfare, supply chain resilience, and environmental conservation."
    3. Bayer announced that the FDA approved AMBELVIST® (gadoquatrane), a new, intravenous macrocyclic gadolinium (Gd)-based contrast agent (mGBCA) indicated for contrast-enhanced magnetic resonance imaging (MRI) to detect and visualize lesions with abnormal vascularity in the central nervous system (CNS) and non-CNS body regions in adult and pediatric patients, including term neonates (15 JUN 2026).[5] The release testing of Ambelvist is validated and registered with PSNG rCR and LAL. AMBELVIST is a next-generation mGBCA with a novel tetrameric structure and high relaxivity (a measure of signal enhancement). This is the first FDA approval for a product to be released using rCR.
- Mitigation of supply chain risk: globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). Growing demand for medicines (thus growing need for BET assays) together with escalating restrictions to harvest horseshoe crabs for biomedical purposes create a supply chain risk.[6] The use of PSNG mitigates this risk.
- Non-animal derived reagent: the use of PSNG reduces global dependency on wild marine arthropods as the raw material for a critical quality control test.
- Sustainable production: PSNG allows for aligning with global sustainability goals and the 3R ethical guidelines (Replacement, Reduction, Refinement).
- Technical advancements:
    1. Documented superior analytical performance: lot-to-lot reproducibility of PSNG (shown on Certificates of Compliance.[7]
    2. Rapid test with Time to Result shorter than LAL assays.[7]
    3. Documented specificity of PSNG to a range of endotoxins (including those with four fatty acid chains within Lipid A structures) and no cross-reactivity with 1,3-beta glucans.[8]
    4. Documented suitability of PSNG with a wide range of products, including lower degree of interference in many products.[8,9]
    5. Documented comparability/equivalency of PSNG to LAL in a variety of matrixes.[6-13]
- Seamless Integration: PSNG is compatible with existing photometric platforms, allowing for straightforward incorporation into existing BET procedures.
- Standard of Quality: PSNG is produced under rigorous cGMP conditions in FDA-licensed facility for manufacture of Limulus Amebocyte Lysate (LAL). PSNG FDA Drug Master File is available for review via US FDA (MF# 031277) – the only Master File for rCR to date.
- Compatibility with automation systems for BET: PSNG was proven to be adopted for high sample throughput productions.
- Technical resources: Access to Global Technical Services (available in local time and languages) regarding any specific implementation concerns and troubleshooting: *techservice@acciusa.com*. Available per request:
    1. Reagent Transfer Protocol
    2. Method Validation Package
    3. Peer-reviewed publications
    4. Contract Test Services

Principle of PyroSmart NextGen®

PSNG contains recombinant Factor C, Factor B and Pro-clotting enzyme co-lyophilized with chromogenic substrate into a reagent.[14] It is kitted together with a reconstitution buffer (different configurations available). As such, PyroSmart NextGen® is a reagent for kinetic chromogenic technique: a quantitative enzymatic assay based on the measure of change in absorbance at 405nm which is proportional to

the dose of bacterial endotoxins present in the sample. The kinetic chromogenic technique is included in USP Chapter <*85*>.[15] Therefore, the stoichiometry and output of PSNG is well understood, published and accepted as a compendial technique. The dynamic range of PSNG is 50 to 0.001 EU/mL, depending on the type of existing absorbance reader.



rCR such as PSNG are included in USP <86> as one of two types of recombinant reagents. Per USP FAQ: <86> Bacterial Endotoxins Test using Recombinant Reagents, *"The Endotoxin Subcommittee to the USP Microbiology Expert Committee (MEC) developed Chapter <86> based on an extensive review of public and submitted confidential end-user data and validations, and the available peer-reviewed literature. Extensive data exists in the peer-reviewed literature for the BET using rFC and rCRs, and the MEC was also able to review non-public/confidential data during the development of the chapter. This information was considered in combination with the recognition that recombinant reagents are the biotechnology equivalent of natural Factor C, the biosensor responsible for endotoxin detection in horseshoe crabs. Additionally, the rCR enzymatic cascade begins with rFC. Combining all of this information and data, the MEC agreed that the inclusion of rCR was supported by the science and data reviewed. Advancing an rBET chapter is aligned with USP's commitment to expanding the use of animal-free methods and materials."*.[16]

PSNG was cloned based on the genome of *Limulus polyphemus.*

Key Differentiators Between LAL and PSNG

Understanding the differences between Limulus Amebocyte Lysate (LAL) and PSNG recombinant cascade reagent is crucial when performing validation studies:

- Source Material: LAL is derived from horseshoe crabs, whereas PSNG is produced through recombinant DNA technology.
- Specificity: PSNG provides specificity to endotoxins only, no cross-reactivity with 1,3-beta glucans due to the absence of Factor G.
- Composition: PSNG formulation is based on decades of research and trials conducted by Seikagaku/ACC endotoxin experts. It reflects the most current knowledge of endotoxin biochemistry. Unlike LAL, PSNG formulation does not contain additional blood-derived proteins and peptides that regulate the blood clotting mechanism in horseshoe crabs.

Status of USP <86>

This current status and upcoming changes of USP <86> are described below:

At the time of writing this article (JUL 2026)

- USP <*86*> is a non-applicable chapter (official on 01 MAY 2025 in USP 2025 Issue 1)
- The methods described in USP <*86*> are considered to be alternative methods to USP <*85*> *Bacterial Endotoxins Test.* Per *USP General Notices and Requirements, 6.30. Alternative and Harmonized Methods and Procedures:* "An alternative method or procedure is defined as any method or procedure other than the compendial method or procedure for the article in question. The alternative method or procedure must be fully validated (see USP <*1225*> *Validation of Compendial Procedures* and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis.".[17,18]

Based on the revisions to water monographs proposed in Pharmacopeial Forum 52,[1] it is projected that as of 25 SEP 2026 with the publication of USP-NF 2027, Issue 2:

- USP <*86*> will become an applicable chapter
- The methods described in USP <*86*> will become compendial for the testing of pharmaceutical waters per 7 USP monographs (Water for hemodialysis, Water for injection, Bacteriostatic water for injection, Sterile water for inhalation, Sterile water for injection, Sterile water for irrigation and Pure steam, official on 01 APR 2027).

After 25 SEP 2026, the methods described in USP <*86*> will remain alternative for samples/products tested per other monographs than the 7 water monographs.

USP Microbiological expert committee is actively working on ways of implementing methods described in USP <*86*> as compendial methods for all products.

STEP 1: User Requirements

Successful implementation of PSNG rCR necessitates a clear understanding of user requirements in order to ensure an effective transition within quality control processes. This is achieved by producing a User Requirement Specification document. Meeting the user requirements will be demonstrated and/or tested as part of the validation protocol.

STEP 2: Equipment, Consumables and Resources Required

Successful implementation of PSNG rCR necessitates a clear understanding of specific user needs that ensure an effective transition within quality control processes:

- Equipment:

Equipment	Available Yes/No
Incubating absorbance microplate reader (e.g. Agilent Epoch 2)	
Absorbance detection at 405nm wavelength	
Current software built with settings needed for PSNG	
A set of pipettes (P20, P100, P1000)	
Repeater or multichannel pipette	
Vortex mixer	

- Consumables: All must be certified free of interfering endotoxins and must be meet the vendor's requirements

Consumables	Available Yes/No
PSNG lot matched with CSE lot (Certificate of analysis available)	
96well microplates (non-treated, non-coated) e.g. ACC Pyroplates*	
Dilution tubes	
Pipette tips	
Repeater Combi-tip (for repeaters) or a reservoir (for multichannel pipette)	
LAL Reagent Water	
*Not all 96well microplates are equal. The validation conditions for PSNG specify the use of non-coated, non-treated microplates together with a low onset OD of 0.03 (= mOD of 30).	

- SOP Transfer: Since the use of PSNG is like-to-like with kinetic photometric assays, existing LAL SOPs can be quickly adopted for use with PSNG.
- Training and Education: Training the analyst on the best practices of reagent handling and assay preparation, emphasizing the differences between the in-house LAL and PSNG reagent.

STEP 3: Risk Analysis

Before proceeding with implementation, conduct a product-specific risk assessment specific to bacterial endotoxin:

- Evaluation of the regulatory barrier for each sample based on their regulatory status (whether monographed or non-monographed)*
- Evaluation of the likelihood of endotoxin contamination (based on historical data sets and manufacturing process)**
- Evaluation of severity of endotoxin contamination**
- Process microflora: corroborating data (i.e. bioburden data and associated identification of the bacteria; Total Organic Carbon data for purified waters) may be used to determine the occurrence of the most sever risk such as false negative results.

The output of the risk analysis is grouping samples into three categories based on the regulatory threshold:

- **No regulatory threshold samples**
- **Low regulatory threshold samples**
- **High regulatory threshold samples**

PSNG may be implemented in a phase-wise process starting with the lowest regulatory threshold samples.

STEP 4: Implementation

No Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with no additional testing requirements. The end user shall follow the general expectations as defined in USP <*86*> under Preparatory Testing and USP <*1085*> under Method Suitability Testing[19] in order to comply with all the scientific principles of the test:

1. Assurance of the criteria for the standard curve when using PSNG per USP <*86*>

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	| R | ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Notes:	1 lot of PSNG	

2. Method suitability testing when using PSNG per USP <*1085*>: must be demonstrated for each product individually

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	| R | ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Product	Series of dilutions not exceeding MVD in duplicate	< Endotoxin limit
Positive Product Controls (PPC)	Series of dilutions spiked with CSE to the middle of standard series in duplicate	PPC recovery % = 50 – 200%
Notes:	1 lot of PSNG 1 lot of product	

**Detailed information can be found in Appendix 1 **Detailed information can be found in Appendix 2*

3. Test for Interfering factors when using PSNG per USP <*86*>: must be demonstrated for each product individually

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	| R | ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Product	A single dilution (< MVD) expected to yield a valid PPC in duplicate	< Endotoxin limit
PPC	A single dilution spiked to the middle of the standard series in duplicate	PPC recovery % = 50 – 200%
Notes:	1 lot of PSNG 3 lots of product	

4. Internal review and approval (no regulatory filing)

Low Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with no additional testing requirements.

1. Confirm that primary method validation of PSNG was executed in accordance with USP <*1225*> *Validation of Compendial Procedures* to ensure the validation status of the method is suitable for its intended purpose. This can be done via review of the PSNG Primary method validation package (request via *techservice@acciusa.com*).
2. Assurance of the criteria for the standard curve when using PSNG per USP <*86*> (same as for no regulatory threshold samples).
3. Method suitability testing per USP <*1085*>: must be demonstrated for each product individually (same as for no regulatory threshold samples).
4. Verify that the method is suitable for the product being tested in accordance with USP <*1226*> *Verification of Compendial Procedures*.[20] Test for Interfering Factors per USP <86> must be performed for each product individually under actual conditions of use (same as for no regulatory threshold samples).
5. *Equivalency testing to the in-house LAL method is not required within the context of USP <86>. However, a proactive review of the in-house approach with the relevant regulatory authority may help determine the regulator's current expectations relevant to the type of product tested.*
6. Internal review and approval.
7. Filing:
   a. Annual reporting only for pharmaceutical waters
   b. Regulatory review and registration for new drug products

High Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with *no additional* testing requirements.

1. Confirm that primary method validation of PSNG was executed in accordance with USP <*1225*> *Validation of Compendial Procedures* to ensure the validation status of the method is suitable for its intended purpose. This can be done via review of the PSNG Primary method validation package (request via *techservice@acciusa.com*).
2. Assurance of the criteria for the standard curve when using PSNG per USP <*86*> (same as for no regulatory threshold samples).
3. Method suitability testing per USP <*1085*>: must be demonstrated for each product individually (same as for no regulatory threshold samples).
4. Verify that the method is suitable for the product being tested in accordance with USP <*1226*> *Verification of Compendial Procedures*. Test for Interfering Factors per USP <*86*> must be performed for each product individually under actual conditions of use (same as for no regulatory threshold samples).
5. *Equivalency testing to the in-house LAL method is not required within the context of USP <86>. However, a proactive review of the in-house approach with the relevant regulatory authority may help determine the regulator's current expectations relevant to the type of product tested.*
6. Internal review and approval.
7. Regulatory filing: Submit the results (after confirming the process with the appropriate review division) – possibly as reduced reporting category Supplement – Changes being affected or Special Report)

Note: If currently using one of the LAL photometric methods for testing specific samples, the method suitability with PSNG is likely to be the same for that sample.

Note 2: If currently using the gel clot test only, prior to Preparatory testing, the end user must install and validate one of the platforms for kinetic chromogenic measure (i.e. absorbance microplate reader powered by appropriate software).

Conclusions

The new USP chapter <*86*> significantly simplifies the implementation and review process for all sample types and pharmaceutical waters in particular. FDA's approvals of multiple products using recombinant reagents (including using rCR)

signals that the transition from LAL to recombinant reagents for BET is no longer a choice, it is an expectation.

Implementing PyroSmart NextGen® rCR for BET is a strategic move towards greater sustainability, reliability, and efficiency in quality control of biopharmaceutical manufacturing.

By following this guide, your organization can seamlessly transition to PSNG, maintaining high standards of accuracy and compliance while meeting your sustainability goals.

Ready to make the switch? Contact our team today to learn more about how PSNG can enhance your endotoxin testing processes (*techservice@acciusa.com*).

Successful implementation of PSNG rCR necessitates a clear understanding of user requirements in order to ensure an effective transition within quality control processes.

Appendix 1 – Sample Types

Globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). Out of the 70 million BET tests, about 75% of all tests are performed on water samples for pharmaceutical purposes. Therefore, transitioning water testing from LAL to recombinant reagents can yield a dramatic reduction in the use of LAL and significantly improve your organization's Sustainability scorecard and Environmental, Social and Governance (ESG) scores.

Table 1. Estimation of typical samples tested in biopharmaceutical manufacturing

Samples	Percentage of sample types
Waters for pharmaceutical purposes	75%
Pharmaceutical ingredients	10%
Buffers and solutions	2%
In-process materials	3%
Finished products	10%

Water for Pharmaceutical Purposes

There are different water samples tested daily for endotoxin and used within biopharmaceutical manufacturing: monographed and non-monographed samples. Monographed samples have their specific monographs listed in the *US Pharmacopeia – National Formulary* (USP-NF) and some of them include the test for Bacterial Endotoxin under the list of specific tests required to be performed along with an endotoxin specification.

The output of the risk analysis is grouping samples into three categories of sample types based on the level of regulatory

Table 2. List of water samples per USP **<1231> Water for Pharmaceutical Purposes** - monographed vs. non-monographed[21]

Samples	USP Monograph?	Reference to USP <85>?	Future Reference to USP <86>?
Purified Water	Yes	No	No
Water for Injection (WFI)	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Water for hemodialysis	Yes	Yes, < 1 EU/mL	Yes, < 1 EU/mL
Pure Steam	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile Purified Water	Yes	No	No
Sterile Water for Injection	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Bacteriostatic Water for Injection	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile water for Irrigation	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile Water for Inhalation	Yes	Yes, < 0.5 EU/mL	Yes, < 0.5 EU/mL
Drinking water	No	N/A	N/A
Distilled water	No	N/A	N/A
Deionized water	No	N/A	N/A
Filtered water	No	N/A	N/A
High-purity water	No	N/A	N/A
Deaerated water	No	N/A	N/A

Table 3. Estimation of probability of endotoxin ingress per sample type based on validated manufacturing process and historical data sets.

Samples	Probability of endotoxin ingress
Water for Injection (WFI)	Very low
Pharmaceutical ingredients	Low
Buffers and solutions	Low
In-process materials	Moderate
Finished products	Low

Table 4. An example of Risk analysis of endotoxin ingress in pharmaceutical manufacturing per sample type

		Severity					
		Hazardous A	High B	Moderate C	Low D	Minor E	Negligible F
Probability	Almost certain 7						
	Very high 6	HIGH RISK					
	High 5			Medium RISK			
	Moderate 4	4B – In-process materials			LOW RISK		
	Low 3	3A – Finished products		3 C – Ingredients			
	Very low 2			2C – WFI	2D – Buffers and solutions		
	Remote 1						

Table 5. Final categories of sample types

Regulatory threshold	Sample types	Comments	Implementation requirements
NO	Non-monographed water samples: • Purified water • Sterile purified water • Drinking water • Distilled water • Deionized water • Filtered water • High purity water • Deaerated water	Low risk	Follow USP <*86*>
LOW	Monographed samples: • WFI, SWFI • Pure steam • Pharmaceutical ingredients, buffers and solutions	Low risk Annual reporting only	Follow USP <86> Reference FDA MF# 031277 for PSNG Equivalency studies should not be needed unless requested by the relevant regulatory authority
	New non-monographed parenteral products	High risk New regulatory review and filing New registration	
HIGH	In-process materials Legacy monographed parenteral products	High risk Re-validation Regulatory review and approval for legacy products Re-registration per country	Follow USP <86> Reference FDA MF# 031277 for PSNG Equivalency studies should not be needed unless requested by the relevant regulatory authority

Globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). Out of the 70 million BET tests, about 75% of all tests are performed on water samples for pharmaceutical purposes.

requirements needed towards implementation of PSNG as shown in Table 5.

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15. *Current USP <85> Bacterial Endotoxins Test*
16. *https://www.uspnf.com/notices/86-alternative-faq*
17. *Current USP General Notices and Requirements*
18. *Current USP <1225> Validation of Compendial Procedures*
19. *Current USP <1085> Guidelines for Bacterial Endotoxins*
20. *Current USP <1226> Verification of Compendial Procedures*
21. *Current USP <1231> Water for Pharmaceutical Purposes*

Additional information available at *www.acciusa.com* or via *techservice@acciusa.com*.