

## BETMAT Recombinant Cascade Reagent (rCR)

### Frequently Asked Questions

#### What are recombinant cascade reagents (rCR)?

Recombinant cascade reagents (rCR) are recombinant protein-based detection systems designed for endotoxin assays, replacing traditional LAL reagents. rCR consists of key recombinant proteins (recombinant Factor C, recombinant Factor B, and recombinant pro-clotting enzyme) from the Limulus coagulation cascade. These proteins are expressed via genetic engineering to replicate the endotoxin-recognition and signal-amplification function of natural LAL.

#### What are the key characteristics of BETMAT rCR?

The key characteristics of BETMAT rCR are:

- High specificity: There is no factor G in the rCR reagent, thus it is an endotoxin-specific reagent that eliminates the risk of false positives from (1→3)  $\beta$ -D-Glucan.
- Stability & consistency: Gene recombinant technology enables BETMAT to produce rCR reagents which avoids batch-to-batch variation of natural LAL.
- Ethical & sustainable: Eliminates the need for using horseshoe crab blood, reducing ecological impact.
- High Sensitive: rCR reagent mimics signal amplification cascade in the factor C pathway, enabling detection of endotoxin using kinetic chromogenic method with a high sensitivity.

### What kind of instrument does it require for performing BETMAT rCR assay?

BETMAT rCR is a kinetic chromogenic assay similar to nature LAL kinetic chromogenic assay. The assay method is fully compatible with existing incubating microplate readers, software, and endotoxin-free accessories for kinetic chromogenic assay. There is no need to inquire about new endotoxin assay instruments for BETMAT rCR assay. This allows for easier integration BETMAT rCR assay by reducing the learning curve, creating cost efficiencies.

### What is the difference between rCR and rFC?

Recombinant factor C (rFC) and recombinant cascade reagents (rCR) are both used for endotoxin assays, but they differ in terms of composition, reaction principles, detection methods, and performance characteristics. The specific differences are as follows:

- **Composition:** rFC contains only a single enzyme, recombinant Factor C, the first enzyme in amoebocytes of horseshoe crab serine protease catalytic cascade activated by endotoxin. In contrast, rCR consists of three enzymes, recombinant Factor C, recombinant Factor B, and recombinant proclotting enzyme, which reconstructs a complete reaction cascade, similar to the composition of natural LAL reagent.
- **Reaction Principle:** When using rFC for detection, endotoxin activates the recombinant Factor C, and the activated Factor C cleaves the fluorescent substrate to produce a fluorescent signal, which is a relatively simple single - step reaction. rCR simulates the enzyme - linked reaction of natural LAL reagent. Endotoxin first activates recombinant Factor C, then sequentially activates recombinant Factor B, and finally converts recombinant proclotting enzyme into clotting enzyme, forming a three - step reaction chain.
- **Detection Method:** rFC usually requires a fluorescence microplate reader for quantitative determination of the fluorescent signal by end point assay. rCR, on the other hand, can be detected by a kinetic absorbance microplate reader, and it



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monitors the signal through color change, which is the same as the detection method of traditional kinetic chromogenic LAL reagent.

- Instrument Requirements: rFC detection requires the use of fluorescence - based instruments. rCR can use the same type of instrument as the traditional LAL reagent, and there is no need to change the detection equipment, which is more convenient for laboratories that originally used traditional methods to transition.

### Is the testing methodology of BETMAT' s rCR different from the kinetic chromogenic LAL assay?

BETMAT designed our rCR reagent using the same testing methodology of nature kinetic chromogenic LAL assay. Users will prepare the rCR assay the same way as the traditional chromogenic LAL assay. The rCR assay uses 100 µl of sample, negative controls, and standards, as well as 100 µl of rCR reagent in each assay well.

### What is the global regulatory status of rCR?

Recombinant cascade reagents are increasingly recognized globally, with some pharmacopoeias having included them as official methods, while others are still in the process of improvement and harmonization. The specific regulatory status is as follows:

- United States: The United States Pharmacopeia (USP) has included recombinant Factor C (rFC) and recombinant cascade reagent (rCR) in USP < 86 > Bacterial Endotoxins Test Using Recombinant Reagents in May 2025. According to the regulations, users are required to verify the applicability of recombinant reagents to specific products.
- Europe: The European Pharmacopoeia (EP) has included the recombinant Factor C method in General Chapter 2.6.32 Test for Bacterial Endotoxins Using Recombinant Factor C. It stipulates that water for injection and purified water can be directly detected by this method, and complex matrix samples need to be used



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after verification. However, currently, EP 2.6.32 only includes the recombinant Factor C method, and has not yet included recombinant cascade reagents.

- Japan: The Japanese Pharmacopoeia lists recombinant protein reagents, includes rFC and rCR as a supplementary method for endotoxin testing through the guideline < G4 - 4 - 180 > Bacterial Endotoxins Test and Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay, which describes the procedures and considerations for using recombinant protein reagents for endotoxin testing.

#### Is validation necessary for all products when implementing BETMAT rCR?

Yes, according to USP chapter ( 86 ) Bacterial Endotoxins Test Using Recombinant Reagents, the users will have to validate recombinant cascade reagent assay method before implementing this new assay. BETMAT provides primary validation package for the users to verify that the recombinant cascade reagent is appropriate for use in testing specific products or materials.