

Catalog Numbers:

EFC005 (96 tests): Assay range 0.005-5 EU/mL

EFC010 (96 tests): Assay range 0.01-10 EU/mL

PRODUCT DESCRIPTION

Recombinant Factor C (rFC) offers an animal-free approach to endotoxin detection and is increasingly recognized as a reliable substitute for traditional Limulus Amebocyte Lysate (LAL) assays that rely on horseshoe crab amebocyte. By removing the need for crab-derived materials, rFC reduces environmental impact and minimizes the variability associated with LAL testing. Native Factor C is the endotoxin-sensing enzyme in horseshoe crab amebocyte and the first enzyme activated in the serine protease cascade. The recombinant form reproduces this natural activity through gene recombination techniques, enabling accurate detection and quantification of bacterial endotoxins in pharmaceutical and biological samples. Once exposed to endotoxin, rFC converts from an inactive proenzyme into an active protease that cleaves a fluorescent substrate, generating a signal proportional to the endotoxin concentration, which can be quantified accurately and reliably. Regulatory authorities, including the U.S. FDA and the European Pharmacopoeia, recognize rFC as a robust alternative to LAL when properly validated, offering improved sensitivity and consistency.

The R&D Systems™ Endotoxin rFC Quantitation Kit provides an optimized, animal-free solution that meets regulatory expectations and ensures precise, highly sensitive, and reproducible endotoxin measurement. The kit uses recombinant expressed *Tachypleus* Factor C to leverage the endotoxin-activated enzymatic mechanism, where rFC detects endotoxin, becomes catalytically active, and cleaves a fluorescent substrate to generate a measurable signal directly proportional to endotoxin levels. Endotoxin concentrations in test samples are then determined by comparison to a standard curve.

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

R&D Systems rFC Endotoxin Test Kit enables *in vitro* quantitative measurement of gram-negative bacterial endotoxins (lipopolysaccharides) through activation of rFC.

Available in two assay ranges: 0.005-5 EU/mL (EFC005) and 0.01-10 EU/mL (EFC010).

Note: The R&D Systems rFC Endotoxin Test Kit (Recombinant Factor C Fluorometric Assay) contains sufficient reagents to perform 96 tests per kit.

OTHER SUPPLIES REQUIRED

- Pyrogen-free pipettes, 1 mL, 100 µL, or automatic pipettes with pyrogen-free tips
- Endotoxin-free glass dilution tubes for endotoxin and sample dilution
- Test tube rack
- Timer
- Vortex mixer
- Incubating Fluorescence Microplate reader

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

COMPONENT	KIT CATALOG #	QUANTITY PER TEST KIT	CAP COLOR	DESCRIPTION	STORAGE OF MATERIAL
Recombinant Factor C (rFC)	EFC005	2 vials	Blue	Co-lyophilized rFC and fluorogenic substrate. Kit sensitivity (λ) is the lowest rFC endotoxin concentration in the standard curve: 0.005 EU/mL (EFC005), 0.01 EU/mL (EFC010). Each vial supports 48 tests.	Store at 2-8 °C. Avoid exposure to temperatures above 25 °C or prolonged bright light.
	EFC010	2 vials			
Reconstitution Buffer	EFC005	2 vials (3 mL/vial)	Silver	For rehydration of lyophilized rFC.	Store at 2-8 °C.
	EFC010	2 vials (3 mL/vial)			
Endotoxin-free Water	EFC005	1 vial (50 mL/vial)	Blue	Endotoxin concentration <0.005 EU/mL. Used for rehydration of the Endotoxin Control Standard, dilution of Endotoxin Standards and samples, and as negative control (blank).	Store at 2-30 °C.
	EFC010	1 vial (50 mL/vial)			
Endotoxin Control Standard	EFC005	2 vials	Red	Lyophilized <i>E. coli</i> (0111:B4) Endotoxin Standard. Reconstitute Endotoxin Control Standard with Endotoxin-free Water. Actual potency printed on the vial label.	Reconstituted Solutions <20 EU/mL are stable for 24 hours at 2-8 °C. Do not freeze.
	EFC010	2 vials			
Pyrogen-free Black 8-well Plate Strips	EFC005	1 pack (15 Strips/pack)	NA	Individually wrapped pyrogen-free 8-well strips, 1 rack.	Store at room temperature.
	EFC010	1 pack (15 Strips/pack)			

SAMPLE COLLECTION AND PREPARATION

All glassware, plastic ware, and diluents contacting samples or reagents must be endotoxin-free. Glassware and other heat-stable apparatus can be depyrogenated in an oven using a validated process; a commonly used minimum time and temperature setting is 60 minutes at 250 °C. Use aseptic techniques throughout. Samples to be tested should be stored under conditions that prevent bacterial activity. Samples may be stored at 2-8 °C for short-term storage (less than 24 hours). For long-term storage, samples should be kept below -10 °C.

The optimal pH range for rFC–Endotoxin reaction is 6-8. Adjust acidic or basic samples to this range using endotoxin-free 0.1 N sodium hydroxide, 0.1 N hydrochloric acid, or endotoxin-free Tris buffer. Always measure the pH of an aliquot of the bulk sample to avoid contaminating the main sample with the pH electrode. Test for and eliminate potential interfering substances as described in the Product Inhibition/Enhancement section.

REAGENT PREPARATION

Allow reagents to reach room temperature before use. Prepare all solutions at room temperature.

Preparation of Endotoxin Standards

Note: Reconstitute Endotoxin Control Standards immediately before use.

1. Reconstitute Endotoxin Control Standard with Endotoxin-free Water using the volume specified in the Certificate of Analysis. Vortex vigorously for 5 minutes to obtain a 10 EU/mL Endotoxin Stock Solution. The reconstituted stock is stable for 24 hours at 2-8 °C. **Do not freeze.**

2a. Endotoxin Low Standard Preparation (0.005-5 EU/mL)

CONCENTRATION	INSTRUCTIONS
5 EU/mL	Mix 0.5 mL of 10 EU/mL Endotoxin Stock Solution with 0.5 mL Endotoxin-free Water, vortex for 1 minute to obtain a 5 EU/mL endotoxin solution.
0.5 EU/mL	Mix 0.1 mL of 5 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.5 EU/mL endotoxin solution.
0.05 EU/mL	Mix 0.1 mL of 0.5 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.05 EU/mL endotoxin solution.
0.005 EU/mL	Mix 0.1 mL of 0.05 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.005 EU/mL endotoxin solution.
Blank	Use 0.5 mL of Endotoxin-free Water.

2b. Endotoxin High Standard Preparation (0.01-10 EU/mL)

CONCENTRATION	INSTRUCTIONS
10 EU/mL	10 EU/mL Endotoxin Stock Solution
1 EU/mL	Mix 0.1 mL of 10 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 1 EU/mL endotoxin solution.
0.1 EU/mL	Mix 0.1 mL of 1 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.1 EU/mL endotoxin solution.
0.01 EU/mL	Mix 0.1 mL of 0.1 EU/mL endotoxin solution with 0.9 mL Endotoxin-free Water, vortex for 1 minute to obtain a 0.01 EU/mL endotoxin solution.
Blank	Use 0.5 mL of Endotoxin-free water

3. Discard the remaining endotoxin dilutions.

SETTING FLUORESCENCE MICROPLATE READER

1. Set the incubator temperature at 37 °C.
2. Set the plate shaking speed at medium speed for 15 seconds before reading.
3. Set the reading wavelength: using a fluorescence microplate reader, set the excitation wavelength to 380 nm and the emission wavelength to 440 nm.

SENSITIVITY SETTING FOR FLUORESCENT READER

Before testing, it is necessary to adjust the sensitivity of the fluorescence readout to ensure the fluorescence signal falls within a suitable range, so that the detection range of the standard curve can be optimized. If the sensitivity is set too low, the fluorescence signal of the point with low endotoxin concentration in the standard curve may be too weak, resulting in difficulty separating the lowest point of the standard curve from the negative control. If the sensitivity is set too high, the fluorescence signal of the point with a high endotoxin concentration in the standard curve may become too strong, exceeding the detection range. Fluorescence signals are normally recorded as Relative Fluorescence Units (RFU). It is recommended to adjust the net Δ RFU value (RFU at time T minus RFU at time zero corrected with the blank) at the point where the endotoxin concentration is 0.5 EU/mL in the standard curve to about 3000 (range from 2000-4500). The sensitivity of the fluorescence reader can be controlled by adjusting the fluorescence gain setting.

Using a microplate, multiple fluorescence gain values should be tested during the initial setup (recommended values: 25, 27, and 29). Measure each concentration point of the standard curve at these settings. Choose the fluorescence gain value that yields a net Δ RFU of approximately 3000 at the 0.5 EU/mL endotoxin concentration point and provides a strong linear correlation coefficient for the standard curve.

TEST PROCEDURE

The following steps must be performed at 37 °C ± 1 °C.

1. Start to run assay after fluorescent microplate temperature-controlled chamber has reached 37 °C.
2. Run assay in duplicate or triplicate.
3. Vortex endotoxin standards and samples before loading.
4. Place the endotoxin-free black microplate strips into the microplate holder. Preheat the microplate for 10 minutes on the microplate reader or dry bath heater before adding reagents.
5. Dispense 100 µL of Endotoxin-free Water (negative control), endotoxin standard solutions, and test sample into the designated wells of the microplate. Avoid introducing air bubbles.
6. After loading, incubate the microplate for 10 minutes on the microplate reader incubator.
7. While the plate is incubating, reconstitute each vial of co-lyophilized rFC and fluorogenic substrate using 2.5 mL of Reconstitution Buffer. Mix gently by tilting and swirling until completely dissolved. **Do not vortex.** Store reconstituted rFC solution at 2-8 °C for up to 2 hours or at -20 °C for up to 1 month.
8. After incubation, remove the microplate and add 50 µL of rFC solution to each well quickly and evenly, avoiding air bubbles. Leave the microplate uncovered.
9. Place the microplate into the fluorescent microplate reader.
10. Start the reading program, ensuring it includes a 15-seconds medium speed shake to mix the rFC and samples.
11. After mixing, read fluorescence signal at time zero.
12. Incubate for the specified time (T) and record fluorescence signal again at elapsed time T, as indicated in the Certificate of Analysis.

DATA COLLECTION AND ANALYSIS

1. Record the RFU values at time T and time Zero.
2. The difference of time T and time Zero RFU readings is Δ RFU.
3. Δ RFU values are corrected with the blank to give net Δ RFU.
4. The log net Δ RFU is then plotted against the log endotoxin concentration to generate a linear regression curve using the following standard curve equation:

$$\log_{10}(Y) = A \times \log_{10} X + B, \text{ where } Y = \text{net } \Delta \text{ RFU (RFU at time T minus RFU at time Zero corrected with the blank),}$$

$X = \text{endotoxin concentration, } A = \text{slope of the regression curve, } B = \text{the Y intercept}$

PERFORMANCE CHARACTERISTICS

The test is valid only when all of the following requirements met.

1. Prepare at least three endotoxin concentrations within the concentration range along with blanks to generate the standard curve. Perform the assay using at least duplicates of each standard endotoxin concentration.
2. The value of the correlation coefficient (r) of the calculated standard curve should be ≥ 0.980 .
3. The endotoxin recovery of the test sample should be 50-200%.
4. The Δ RFU value (RFU at time T minus RFU at time Zero) at the lowest endotoxin concentration of the standard curve should be greater than the Δ RFU of the negative controls.
5. The %C.V. of Δ RFU for the replicates should be less than or equal to 20% (except for C.V. value of the negative controls). To calculate %C.V., divide the standard deviation of Δ RFU (RFU at time T minus RFU at time Zero) by the mean and express it as a percentage.

ROUTINE TESTING

For routine testing, prepare a series of endotoxin standards and assay them alongside the unknown samples under the same conditions. Determine the endotoxin concentration in the unknowns by comparing performance to the standards. Each standard and unknown sample should be tested in at least duplicate, and the average absorbance of all replicates should be used for calculations. A Positive Product Control (PPC) may be included to monitor for product inhibition or enhancement.

INITIAL QUALIFICATION

As recommended in the Pharmacopeia, validation of the Endotoxin rFC assay should be performed when conditions that are likely to influence the test result change.

Standard Curve Validation. Upon receipt of a new lot of rFC, a standard curve validation test must be performed. Prepare at least three endotoxin concentrations within the standard curve range indicated by the Certificate of Analysis. Perform the assay using at least three replicates of each standard endotoxin concentration. Do not average the Δ RFU of all replicates. The Δ RFU value of the correlation coefficient (r) must be ≥ 0.980 for the endotoxin concentration range tested.

Test for Interfering Factors. The test for interfering factors must be repeated whenever conditions that could affect the test results change. This includes, but is not limited to, changes in test sample formulations, or switching to a new rFC provider. Refer to the Product Inhibition/Enhancement section for details.

PRODUCT INHIBITION/ENHANCEMENT

If there is a potential that the sample contains interfering substances, a recovery rate test should be performed. Prepare a Positive Product Control (PPC), which is a sample spiked with a known amount of endotoxin. The spike concentration of endotoxin (λm) should be in the middle of the standard curve range.

1. Analyze the spiked sample (PPC) along with the unspiked sample.
2. Determine the endotoxin concentration in spiked test sample (C_s) and in unspiked test sample (C_t).
3. Calculate the recovery rate (R): $R = (C_s - C_t) / \lambda m \times 100\%$.
4. Recovery rates within the range of 50-200% suggest non-significant interference. Recovery rates outside the range of 50-200% suggest significant interference. Dilution and modification are commonly employed to reduce the interference to a non-significant level.
5. If R is outside the 50-200% range, repeat the inhibition test using a series of test sample dilutions, ensuring that the dilution does not exceed the Maximum Valid Dilution (highest sample dilution at which endotoxins can still be reliably detected).
6. For routine endotoxin concentration assays, it is best to select a test sample dilution at which R is closest to 100%.

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