# Recombinant Factor C Endotoxin Detection Kit, 96 Tests

**Cat. No.** KITG-013 **Lot. No.** (See product label)

## SPECIFICATION

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| **Product Overview** | The Recombinant C Factor Endotoxin Detection Kit is an endotoxin determination product that does not depend on animal-derived components, providing stable and continuous supply as an alternative to horseshoe crab reagents. It features high sensitivity and strong specificity, with the following characteristics: Does not depend on animal-derived components, providing higher supply security. Endpoint fluorescence determination, comparable to other quantitative LAL methods. Sensitivity range from 0.005 to 5 EU/mL. Endotoxin specificity, no G factor bypass interference. Eliminates dependence on animal-derived reagents, conforming to the 3R replacement principle. Recombinant expression production, good batch-to-batch consistency. Liquid reagent, easy to use. This kit can replace traditional horseshoe crab reagents for endotoxin detection of raw and auxiliary materials, intermediate products, and released products for human and veterinary injectable drugs (such as chemical drugs, radioactive drugs, antibiotics, biological products, etc.) and medical devices (such as dialysis solutions, implantable devices, etc.). |
| **Description** | The Recombinant C Factor Endotoxin Detection Kit is an endotoxin determination product that does not depend on animal-derived components, providing stable and continuous supply as an alternative to horseshoe crab reagents. It features high sensitivity and strong specificity, with the following characteristics: Does not depend on animal-derived components, providing higher supply security. Endpoint fluorescence determination, comparable to other quantitative LAL methods. Sensitivity range from 0.005 to 5 EU/mL. Endotoxin specificity, no G factor bypass interference. Eliminates dependence on animal-derived reagents, conforming to the 3R replacement principle. Recombinant expression production, good batch-to-batch |

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| | consistency. Liquid reagent, easy to use. This kit can replace traditional horseshoe crab reagents for endotoxin detection of raw and auxiliary materials, intermediate products, and released products for human and veterinary injectable drugs (such as chemical drugs, radioactive drugs, antibiotics, biological products, etc.) and medical devices (such as dialysis solutions, implantable devices, etc.). |
| **Form** | Endotoxin Detection Kit |
| **Bio-activity** | Sensitivity range: 0.005 to 5 EU/mL. Endpoint fluorescence determination. Endotoxin specificity, no G factor bypass interference. |
| **Applications** | Endotoxin detection of raw and auxiliary materials, intermediate products, and released products for human and veterinary injectable drugs (chemical drugs, radioactive drugs, antibiotics, biological products, etc.) and medical devices (dialysis solutions, implantable devices, etc.) |
| **Notes** | Kit Components: Endotoxin Working Standard: Lyophilized powder, potency and reconstitution volume see label Fluorescent Substrate Solution: 6 mL/vial × 1 Assay Buffer: 5 mL/vial × 1 rFC Enzyme Solution: 0.6 mL/vial × 2 LAL Reagent Water: 30 mL/vial × 1 Note: This kit contains sufficient reagents for 96 tests (96-well plate: 100 µL/well/test). Required Consumables and Equipment: Disposable pyrogen-free ampoules (for endotoxin working standard dilution) Single-channel and multi-channel pipettes Pyrogen-free, low-retention pipette tips Disposable sterile pyrogen-free 96-well white plates Sterile pyrogen-free reagent reservoirs Vortex mixer Timer Microplate reader capable of fluorescence detection (wavelength set to 380/440 nm) Operation Introduction: The assay is performed in a 96-well plate. After sample addition, fluorescence values are measured at time zero and after incubation at 37°C ± 1°C for one hour using 380/440 nm excitation/emission wavelengths. The net fluorescence difference is obtained by calibrating the fluorescence reading difference (ΔRFU) of working standards and samples with the negative control fluorescence |

difference (ΔRFU). The logarithm of the net fluorescence difference is proportional to the logarithm of endotoxin concentration, and is linear within the range of 0.005 to 5.0 EU/mL. The endotoxin concentration in samples can be calculated according to the standard curve. The kit operation procedure is as follows: Step 1: Reconstitute endotoxin working standard Step 2: Prepare serial endotoxin standard solutions Step 3: Add samples Step 4: Prepare substrate reagent Step 5: Add substrate reagent Step 6: Fluorescence reading Fluorescence signals are usually recorded as Relative Fluorescence Units (RFU). Since the actual fluorescence signal is converted to an electronic signal, which can be adjusted using gain settings or sensitivity settings, RFU is an arbitrary unit. According to the detected signal intensity, the instrument can be adjusted to higher gain/sensitivity settings to enhance weak signals, or to lower gain/sensitivity settings when the signal is too strong. In rFC endotoxin detection assays, log(ΔRFU) corresponds to log(endotoxin concentration EU/mL). If sensitivity is set too low, it will be difficult to detect fluorescence at the lowest standard; if sensitivity is set too high, fluorescence at the highest standard will exceed the detection range. Therefore, before testing, appropriate detection sensitivity must be determined. For example, the fluorescence range of microplate reader is 0-99999. To ensure all points fall within the detection range, the RFU range for 0.5 EU/mL should be 1000-10000, corresponding to a log RFU range of 3-4, with a logarithmic midpoint of 3.5. Therefore, the target absolute net RFU for 0.5 EU/mL is approximately 3000 RFU. To maintain sufficient spacing between the negative control and the lowest standard in routine testing, 3000 RFU can be regarded as the minimum value for 0.5 EU/mL endotoxin concentration. Note: Due to software differences among different brands of microplate readers, users are advised to refer to the microplate reader user manual for instructions. Sample Preparation: 1. Test samples should be handled carefully to avoid microbial or endotoxin contamination. All materials in direct contact with samples or test reagents must be pyrogen-free. Sample dilution should be performed in pyrogen-free ampoules using LAL reagent water. If not tested promptly, samples should be stored refrigerated or frozen. 2. Since rFC endotoxin

determination reagent is an enzymatic reaction for endotoxin concentration determination, the sample pH must be maintained between 6-8. If the sample pH is not within this range, pyrogen-free sodium hydroxide, hydrochloric acid, or other buffer solutions should be used for adjustment. Note: Test samples should not be directly subjected to pH adjustment to avoid false positives caused by endotoxin contamination from pH electrodes. Preliminary experiments must be conducted to determine appropriate pH adjustment methods. 3. Samples that do not interfere with (enhance or inhibit) endotoxin detection can be used directly without dilution. Samples that interfere with endotoxin detection need to be diluted to concentrations that do not interfere with detection. The dilution process should involve thorough vortex mixing for ≥2 minutes. Endotoxin detection interference is usually caused by excessively high concentrations of components in the sample. Therefore, in most cases, appropriate dilution can overcome interference with endotoxin detection. 4. Before using this kit to detect samples, suitability studies must be conducted. Determine whether samples interfere with detection by examining the spike recovery rate of endotoxin detection. If samples are found to interfere with detection, further pretreatment of such samples is required (pH adjustment, dilution, etc.) until interference is overcome. A spike recovery rate between 50-200% is considered as no interference with detection. Examples of interference determination are as follows: Sample Dilution Factor Table: Dilution factor 10: Unspiked 0.083 EU/mL, Spiked 0.106 EU/mL, Difference 0.023, Recovery rate 4.6%, Interference: Severe interference, Actual endotoxin concentration: Unknown Dilution factor 20: Unspiked 0.048 EU/mL, Spiked 0.284 EU/mL, Difference 0.236, Recovery rate 47.2%, Interference: Interference, Actual endotoxin concentration: Unknown Dilution factor 40: Unspiked 0.027 EU/mL, Spiked 0.497 EU/mL, Difference 0.47, Recovery rate 94%, Interference: No interference, Actual endotoxin concentration: 1.08 EU/mL Note 1: Spiked endotoxin concentration is 0.5 EU/mL. Note 2: Spike recovery rate % = (Endotoxin concentration spiked - Endotoxin concentration unspiked) / Spiked endotoxin concentration × 100%. 5. Sample dilution factor should be less than the maximum valid dilution factor of the sample. The

maximum valid dilution factor (MVD) is calculated using the following formula, where the endotoxin limit is the maximum acceptable endotoxin concentration in undiluted sample, and the assay sensitivity is the lowest detection limit of the assay reagent (the lowest detection limit of this kit is 0.005 EU/mL): MVD = Endotoxin Limit (EU/mL) / Assay Sensitivity (0.005 EU/mL) Reconstitution of Endotoxin Working Standard: 6. Add the corresponding volume of LAL reagent water to the endotoxin working standard (hereinafter referred to as CSE) lyophilized powder according to the product label to obtain a solution with concentration of 20 EU/mL. Vortex mix for ≥15 minutes. 7. Users are advised to follow the product instructions provided by the endotoxin working standard manufacturer, and dilute the standard to 5 EU/mL as the starting point. 8. After reconstitution, the endotoxin working standard must be mixed for 15 minutes. Each subsequent dilution step must be mixed for 30 seconds. Pipettes or pipette tips used during the test procedure need to be rinsed and blown dry 3 times. Note 1: Use new pipette tips for each pipetting step to avoid contaminating remaining reagents. Note 2: Unused endotoxin working standard after preparation can be stored at 2-8°C for two weeks. Note 3: For refrigerated endotoxin working standard after preparation, remove and vortex mix for ≥15 minutes before use, and equilibrate to room temperature before use for detection. Note 4: This product cannot be used in combination with other bacterial endotoxin working standards. Preparation of Endotoxin Working Standard Solutions: Take 4 disposable pyrogen-free ampoules, and mark the endotoxin working standard solution concentrations on the vial body (0.005, 0.05, 0.5, and 5 EU/mL respectively). Prepare serial endotoxin standard solutions using the reconstituted 20 EU/mL endotoxin working standard solution. The recommended dilution method is as follows: Endotoxin Standard Concentration 5.0 EU/mL: LAL reagent water 0.75 mL, Endotoxin working standard 0.25 mL of 20 EU/mL solution Endotoxin Standard Concentration 0.5 EU/mL: LAL reagent water 0.9 mL, Endotoxin working standard 0.1 mL of 5 EU/mL solution Endotoxin Standard Concentration 0.05 EU/mL: LAL reagent water 0.9 mL, Endotoxin working standard 0.1 mL of 0.5 EU/mL solution Endotoxin Standard Concentration 0.005 EU/mL: LAL reagent water 0.9 mL, Endotoxin working standard 0.1

mL of 0.05 EU/mL solution Note 1: Since endotoxin adsorbs to plastic tube walls, do not use plastic tubes for endotoxin dilution. Note 2: Before dilution, the previous concentration endotoxin working standard solution used for dilution should be thoroughly vortex mixed (1400 rpm) for ≥2 minutes. For example, when preparing 5 EU/mL endotoxin standard, first vortex mix the 20 EU/mL endotoxin working standard solution for ≥2 minutes; when preparing 0.05 EU/mL endotoxin standard, first vortex mix the 0.5 EU/mL endotoxin working standard solution for ≥2 minutes. Note 3: Diluted endotoxin standard solutions are stable for 8 hours at 2-8°C; discard after 8 hours. Sample Addition to Assay Plate: 1. Add 100 µL of negative control (LAL reagent water), endotoxin working standard solutions, and samples (two groups) respectively to the corresponding wells of a 96-well sterile pyrogen-free white plate. Usually two or three replicate wells are needed. Add 10 µL of 5 EU/mL endotoxin working standard solution to one group of sample wells as the spiked sample group. 2. Incubate the 96-well plate after sample addition in a 37°C ± 1°C incubator for 10-20 minutes. Note 1: Each sample test must be performed simultaneously with endotoxin working standard solution testing, and a new standard curve must be plotted. Note 2: It is recommended to spike each sample. Preparation of Substrate Reagent: Prepare substrate reagent during the 96-well plate preheating period: 1. Before preparing substrate reagent, remove fluorescent substrate solution, assay buffer, and rFC enzyme solution 30 minutes in advance to allow all reagents to equilibrate to room temperature. 2. Prepare substrate reagent by mixing fluorescent substrate solution, assay buffer, and rFC enzyme solution in a 5:4:1 ratio. Mix gently and thoroughly, avoiding vortex mixing. The preparation volumes corresponding to the number of assay wells are as follows: Assay Wells: 12 - Fluorescent substrate solution: 800 µL, Assay buffer: 640 µL, rFC enzyme solution: 160 µL, Total: 1600 µL Assay Wells: 24 - Fluorescent substrate solution: 1400 µL, Assay buffer: 1120 µL, rFC enzyme solution: 280 µL, Total: 2800 µL Assay Wells: 36 - Fluorescent substrate solution: 2000 µL, Assay buffer: 1600 µL, rFC enzyme solution: 400 µL, Total: 4000 µL Assay Wells: 42 - Fluorescent substrate solution: 2300 µL, Assay buffer: 1840 µL, rFC enzyme

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solution: 460 µL, Total: 4600 µL Assay Wells: 48 - Fluorescent substrate solution: 2600 µL, Assay buffer: 2080 µL, rFC enzyme solution: 520 µL, Total: 5200 µL Assay Wells: 54 - Fluorescent substrate solution: 2900 µL, Assay buffer: 2320 µL, rFC enzyme solution: 580 µL, Total: 5800 µL Assay Wells: 60 - Fluorescent substrate solution: 3200 µL, Assay buffer: 2560 µL, rFC enzyme solution: 640 µL, Total: 6400 µL Assay Wells: 66 - Fluorescent substrate solution: 3500 µL, Assay buffer: 2800 µL, rFC enzyme solution: 700 µL, Total: 7000 µL Assay Wells: 72 - Fluorescent substrate solution: 3800 µL, Assay buffer: 3040 µL, rFC enzyme solution: 760 µL, Total: 7600 µL Assay Wells: 78 - Fluorescent substrate solution: 4100 µL, Assay buffer: 3280 µL, rFC enzyme solution: 820 µL, Total: 8200 µL Assay Wells: 84 - Fluorescent substrate solution: 4400 µL, Assay buffer: 3520 µL, rFC enzyme solution: 880 µL, Total: 8800 µL Assay Wells: 90 - Fluorescent substrate solution: 4700 µL, Assay buffer: 3760 µL, rFC enzyme solution: 940 µL, Total: 9400 µL Assay Wells: 96 - Fluorescent substrate solution: 5000 µL, Assay buffer: 4000 µL, rFC enzyme solution: 1000 µL, Total: 10000 µL Note 1: Add fluorescent substrate solution, assay buffer, and rFC enzyme solution sequentially to the preparation container, ensuring rFC enzyme solution is added last. Note 2: Substrate reagent should be prepared fresh and used immediately. Do not prepare excessive amounts at once. Addition of Substrate Reagent and Fluorescence Detection: 1. Set microplate reader detection parameters as follows: Key Parameters and Settings: Excitation wavelength: 380 nm Emission wavelength: 440 nm Optical position: Top Scans per well: 6 Delay before scan (milliseconds): 150 Delay between scans (milliseconds): 1 Shaking: Shake for 15 seconds before reading 2. Example parameter settings for microplate reader: Optical Configuration: Monochromator Read Modes: FL Read Type: Endpoint Wavelengths - Excitation: 380 nm, Emission: 440 nm Plate Type: 96 wells PMT and Optics - PMT Gain: Automatic, Flashers per read: 6, Read From TOP: 1 mm, Read Height: Row, Read Order: Row 3. Remove the preheated 96-well plate, and use a multi-channel pipette to add substrate reagent to all sample wells, 100 µL/well. 4. Immediately read the fluorescence value at time zero after substrate reagent addition. 5. Place the

microplate in a 37°C ± 1°C incubator, and read the fluorescence value at the one-hour time point after incubation for one hour. Data Analysis: 1. Export the fluorescence values read by the microplate reader (time zero and one-hour time point) to an electronic spreadsheet. 2. Subtract the time zero fluorescence value from the one-hour time point fluorescence value for all wells (ΔRFU). 3. Subtract the ΔRFU of the negative control well from the ΔRFU data of standards and samples to obtain the net ΔRFU values of standards and samples. 4. Calculate the logarithm of the average net ΔRFU and concentration (EU/mL) of the endotoxin working standards. 5. Plot with the logarithm of endotoxin working standard solution concentration as the x-axis and the logarithm of net ΔRFU as the y-axis, and perform linear fitting: log(ΔRFU) = A*log(Endotoxin concentration EU/mL) + B. The correlation coefficient $R^2$ of the standard curve should be ≥0.980. 6. Calculate the endotoxin concentration of samples and spiked samples, and calculate the spike recovery rate (Spike recovery rate % = (Endotoxin concentration spiked - Endotoxin concentration unspiked) / Spiked endotoxin concentration 0.5 EU/mL × 100%). If the spike recovery rate is between 50%-200%, the sample is considered to have no interference with detection, and the detection result is reliable. 7. For samples with qualified spike recovery rates, calculate the endotoxin concentration of undiluted samples according to the sample dilution factor. Note: For standard curve linear fitting and calculation of unknown sample endotoxin concentrations, manual calculation can be used, or the microplate reader's own software can be fully utilized to automatically calculate each detection by setting up a new detection template. Precautions: This kit is only for in vitro endotoxin detection of samples and cannot be used for clinical diagnosis and treatment. All consumables in contact with assay reagents during endotoxin detection must be confirmed as sterile and pyrogen-free products to avoid contaminating assay reagents. Before use, allow all reagents to equilibrate naturally to room temperature (20-25°C). Use low-retention pipette tips. Use appropriately calibrated pipettes to ensure accurate transfer of all small-volume liquids as much as possible. Reagents from different batches must not be mixed for use.

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| **Storage** | This kit is shipped with ice packs. Upon receipt, please store immediately at 2-8°C. The unopened product has a shelf life of 12 months. After reconstitution, the endotoxin working standard should be stored at 2-8°C with a validity period of two weeks. |
| **Shipping** | Shipped with ice packs. |
| **Usage** | For in vitro endotoxin detection of samples only. Cannot be used for clinical diagnosis and treatment. |

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