Recombinant Factor C Endotoxin Detection Kit

Cat.No.: HYI131

Product Description

Recombinant factor C endotoxin detection kit can replace the traditional limulus amebocyte lysate (LAL) for human and animal injection drugs (such as chemicals, radiopharmaceuticals, antibiotics, biological products, etc.) and medical devices (such as dialysate, implantable devices, etc.) raw and auxiliary materials, intermediate products, released products endotoxin detection. This kit is a highly sensitive, highly specific, stable and sustainably available alternative to limulus amebocyte lysate that does not depend on animal-derived components. It can measure endotoxin concentrations in the range of 0.005-5 EU /mL.

Detection principle

Limulus factor C is a serine proteinogen sensitive to bacterial endotoxin in limulus blood cells. Limulus factor C is the first to be activated by endotoxin in bacterial endotoxin-mediated limulus blood agglutination system to initiate the hemagglutination cascade in limulus blood cells. Recombinant Factor C is a recombinant factor C (rFC) expressed in the form of gene recombination. The recombinant factor C, which is bound and activated by endotoxin, can cut the substrate to obtain free fluorophores. The release of fluorophores is proportional to the concentration of endotoxin, so that endotoxin can be quantitatively detected. Compared with the classical limulus amebocyte lysate endotoxin detection method, recombinant factor C endotoxin detection method has higher specificity, better specificity, precision, accuracy, linear range and limit of quantitation, and is an improved method for limulus amebocyte lysate endotoxin detection.

Components

Reagent	48T	96T
Fluorescent substrate solution	3 mL	6 mL
Measuring buffer	2.5 mL	5 mL
rFC enzyme solution	0.6 mL	1.2 mL
LAL reagent water	30 mL	30 mL

Control standard endotoxin	1 vial	2 vials
96-well non-pyrogenic detachable reaction plate	1 Pc	1 Pc

Note: Control standard endotoxin is freeze-dried powder. See label for titer and reconstitution volume.

Transportation and storage methods

2~8℃ transport. Upon receipt of the kit, store it at 2~8℃ immediately. Unopened kits are valid for 12 months. The control standard endotoxin is stored at 2~8 ℃ after dissolution, and the validity period is up to 8 weeks.

Operation brief

The assay was performed in a 96-well plate, and the fluorescence values were measured at an excitation/emission wavelength of 380/440nm at zero hour after sample addition and one hour after incubation at 37°C ±1°C. The net fluorescence difference is obtained by calibrating the fluorescence reading difference (ΔRFU) between the control standard endotoxin and the sample using the fluorescence difference (ΔRFU) of the negative control. The logarithm of the net fluorescence difference is proportional to the logarithm of the endotoxin concentration and is linear over the range 0.005 to 5.0EU/mL. The endotoxin concentration in the sample can be calculated according to the standard curve.

Required consumables and equipment

Disposable pyrogen free glass dilution tube (for control standard endotoxin dilution); Sterile pyrogen free pipettes and suction heads; Vortex oscillator; A timer; A fluorescent reader instrument capable of fluorescence detection (wavelength set to 380nm/440nm).

Instructions

1. Preparation before experiment

■ **Sample preparation**

1) The sample to be tested should be handled carefully to avoid microbial or endotoxin contamination. All materials in direct contact with the sample or test reagent should be pyrogen free; Sample dilution should be carried out in a pyrogen glass dilution tube with LAL reagent water. If not detected in time, it is recommended to refrigerate or freeze.
2) The pH value of the sample to be tested should be maintained between 6-8. If the pH of the sample is not in this range, it needs to be adjusted with endotoxin-free sodium hydroxide,

hydrochloric acid, or other buffer solutions. Note: The sample to be tested can not be directly adjusted to the pH, so as not to be contaminated by the pH electrode endotoxin resulting in false positives. It is necessary to pre-test to investigate the appropriate pH adjustment method.

3) Samples that do not interfere with (enhance or inhibit) endotoxin detection can be directly used for detection without dilution; The samples that interfere with the detection of endotoxin should be diluted to a concentration that does not interfere with the detection, and the dilution process should be full of whirlpool oscillation ≥ 1min. Usually, interference with endotoxin detection is due to the high concentration of components in the sample, so in most cases, interference with endotoxin detection can be overcome by moderate dilution.
4) Before using the kit to test the sample, it is necessary to conduct applicability research. Whether the sample interferes with the test is determined by examining the recovery rate of the sample for endotoxin detection. If the sample interferes with the test, the sample needs to be further pretreated (adjusting pH, dilution, etc.) until the interference is overcome.
5) The dilution ratio of the sample should be less than the maximum effective dilution ratio of the sample, the maximum effective dilution ratio of the sample (MVD) is calculated as follows: MVD= Endotoxin Limit (EU/mL)/Assay sensitivity (0.005 EU/mL), where the endotoxin limit is the maximum acceptable concentration of endotoxin in an undiluted sample, and assay sensitivity is the minimum detection limit for assay reagents (the minimum detection limit for this kit is 0.005 EU/mL).

■ **Sensitivity setting of the fluorescent reader instrument**

1) The fluorescence signal is usually recorded as a relative fluorescence unit (RFU). Since the real fluorescent signal is converted into an electronic signal that can be adjusted using either a gain setting or a sensitivity setting, the RFU is an arbitrary unit. Depending on the detected signal strength, the instrument can be adjusted to a higher gain/sensitivity setting to enhance a weak signal, or the instrument can be turned down to a lower gain/sensitivity setting if the signal is too strong. In the test of endotoxin detection by rFC method, log(ΔRFU) corresponds to log (endotoxin concentration EU/mL), if the sensitivity is too low, it will be difficult to detect the minimum standard fluorescence; If the sensitivity is adjusted too high, the highest standard fluorescence will be out of the detection range. Therefore, before conducting the test, the appropriate detection sensitivity must be determined. For example, BioTeck Synergy H1 has a fluorescence range of 0-99999. In order for the fluorescence of all points to fall within the detection range, the RFU range of 0.5 EU/mL should be 1000-10000, corresponding to the logarithmic range of RFU 3-4, and the logarithmic midpoint is 3.5. Therefore, the target absolute

net RFU of 0.5 EU/mL is approximately 3000 RFU. In order to maintain a sufficiently large gap between the negative control and the minimum standard in routine detection tests, 3000 RFU can be considered as the minimum concentration of 0.5 EU/mL endotoxin. Note: Due to the differences in the software carried by different brands of fluorescent reader instrument, users are advised to refer to the instructions in the user manual of fluorescent reader instrument.

2. Detection steps

■ **Dissolution of control standard endotoxin**

1) Add the corresponding volume of LAL reagent water to the lyophilized powder according to the product label to obtain a solution of 20EU/mL, and swirl for ≥15min;

Note: ①Use a new pipette suction for each pipette step to avoid contaminating the remaining reagent; ②The control standard endotoxin refrigerated after preparation should be balanced to room temperature and swirled for ≥15min before being used for testing.

■ **Prepare control standard endotoxin solution**

1) Take 4 disposable pyrogen free glass dilution tubes, mark the concentration of control standard endotoxin solution (0.005, 0.05, 0.5 and 5 EU/mL, respectively) on the tube, and a series of control standard endotoxin solutions are prepared with 20EU/mL of the control standard solution of endotoxin. It is recommended to dilute the control standard endotoxin by gradient dilution method, as shown in the following table.

Concentrations of	The addition of	The addition of control
5.0	0.75	0.25mL 20EU/mL solution
0.5	0.9	0.1mL 5EU/mL solution
0.05	0.9	0.1mL 0.5EU/mL solution
0.005	0.9	0.1mL 0.05EU/mL solution

Note: ①Because endotoxin will be adsorbed on the plastic tube wall, do not use plastic tube to dilute endotoxin; ②Before dilution, the control standard endotoxin solution of the previous concentration of endotoxin for dilution should be sufficiently swirled (1400rpm) for≥1min. For example, when preparing the 5 EU/mL control standard endotoxin, the 20EU/mL control standard endotoxin solution should be sufficiently swirled for≥1min; When preparing 0.05 EU/mL endotoxin standard, the 0.5 EU/mL control standard endotoxin solution should be swirled for≥1min. ③ Diluted control standard endotoxin solution should be used as soon as possible.

■ **Add sample to test plate**

1) 100μl negative control (LAL reagent water), control standard endotoxin solution, and sample (two groups) were added to the corresponding hole of the 96-well sterile pyrogen free plate, usually requiring two or three multiple holes. In one of the two groups of sample holes, 10 μl 5 EU/mL of control standard endotoxin solution was added as the spiked sample group.

Note: ①Each sample test should be performed at the same time control standard endotoxin solution test, draw a new standard curve; ②It is recommended that each sample be spiked.

■ **Prepare substrate reagent**

1) Substrate reagents can be prepared at the same time during the preparation of control standard endotoxin solution: Before the preparation of substrate reagents, remove the fluorescent substrate solution, determination buffer solution and rFC enzyme solution 30min in advance, so that the temperature of each reagent is balanced to room temperature;
2) Prepare the substrate reagent, mix the fluorescent substrate solution, determination buffer and rFC enzyme solution in the ratio of 5:4:1, gently and fully mix, do not swirl mixing.

Note: ①Add the substrate reagent to the preparation container in the sequence of absorbing the fluorescent substrate solution, measuring buffer solution and rFC enzyme solution, and ensure that the rFC enzyme solution is added last; ②Substrate reagent please use now, do not prepare too much at one time.

■ **Add substrate reagent and fluorescence detection**

1) Set the detection parameters of the fluorescent reader instrument: excitation wavelength 380nm, emission wavelength 440nm;
2) Use a pipette to add substrate reagent to all sample holes, 100μl/hole, pay attention to change the suction head when pipetting, to avoid contaminating the remaining reagent;
3) After the substrate reagent is added, the fluorescence value of zero hour at the time point is read immediately;
4) The 96-well plate was placed in an incubator at 37°C±1°C, and the fluorescence value was read for one hour at the time point after incubation for one hour.

■ **Data analysis**

1) Export the fluorescence values read by the fluorescent reader instrument (time point zero hour and time point one hour) to the spreadsheet;
2) For all holes, the fluorescence value at time point one hour minus the fluorescence value at time point zero hour (ΔRFU);
3) Subtract the ΔRFU of the negative control hole from the ΔRFU data of the standard and sample to obtain the net ΔRFU values of the standard and sample;

4) Calculate the logarithm of the mean net ΔRFU and concentration (EU/mL) of the control standard endotoxin;
5) The logarithm of the concentration of the control standard endotoxin solution is the horizontal coordinate, and the logarithm of the net ΔRFU is the vertical coordinate plot and linear fitting, log(net ΔRFU)=A*log(endotoxin concentration EU/mL)+B, the value of the correlation coefficient R 2 of the standard curve should be ≥0.980，and slope A ranges from 0.8 to 1.1.
6) Calculate the concentration of endotoxin in the sample and the spiked sample, and calculate the spiked recovery rate of the sample %= (spiked concentration of endotoxin - no spiked concentration of endotoxin) / 0.5 EU/mL×100% of the spiked concentration of endotoxin. If the spiked recovery rate is between 50%-200%, the sample is considered to have no interference with the detection and the detection results are reliable;
7) For the sample with qualified recovery rate, the endotoxin concentration of the undiluted sample was calculated according to the dilution ratio of the sample.

Limit of detection

Determination range: 0.005-5 EU/mL.

Notes

1. Recombinant factor C endotoxin detection kit is only used for in vitro endotoxin detection of samples, and cannot be used for clinical diagnosis and treatment;
2. Consumables in contact with assay reagents during endotoxin detection must be clearly sterile and heat-free raw products to avoid contaminating assay reagents;
3. Before use, let all reagents balance naturally to room temperature (20-25℃);
4. Use a low adsorption pipette head, and use a new pipette head each time the pipette step is operated to avoid contaminating the remaining reagent;
5. The use of appropriate calibrated pipettes, as far as possible to ensure the accurate transfer of all small volumes of liquids;
6. Different batches of reagents shall not be mixed for use.

Common problems and solutions

1. Possible causes of no signal in sample detection and corresponding solutions: ①pipette error: re-detection; ②Interference components: the pre-experiment or the spiked recovery rate of each

experiment is unqualified, indicating interference, diluting the sample or eliminating interference; ③Inappropriate pH: Test the pH value of the sample and adjust it tothe neutral range of 6-8.

2. Possible causes of low detection signal strength and corresponding solutions: ①instrument sensitivity (gain) is too low: improve sensitivity, need higher gain; ②Error in parameter setting of the fluorescent reader instrument: check the parameter setting of the operating instrument; ③ Incubation temperature is too high/too low: check and calibrate temperature; Reagent failure (transportation or storage) or expiration: check storage conditions and packaging materials; Contact technical services to use a new kit or reagent.
3. Possible causes of high detection background values in standard and negative control Wells and corresponding solutions: contamination of assay components (substrate reagent, assay buffer or rFC enzyme solution): Use newly opened reagents, and be sure to use sterile pyrogen free suction heads.
4. Possible causes of poor linearity of standard curve and corresponding solutions: ①Dilution error of control standard endotoxin solution: re-precise dilution; ②The low fluorescence detection sensitivity of the fluorescent reader instrument causes the fluorescence value at 5 EU/mL to exceed the detection range of the instrument: replace the fluorescent reader instrument with a high fluorescence detection sensitivity.