Portelite™ Rapid Fluorimetric Endotoxin Detection Kit

Catalog number: 60008, 60009
Unit size: 50 Tests, 500 Tests

Component	Storage	Amount (Cat No. 60008)	Amount (Cat No. 60009)
Component A: Endotoxin Green™	Desiccated, Freeze (< -15 °C), Minimize light exposure	1 vial	1 vial
Component B: Endotoxin-Free Water	Freeze (< -15 °C)	1 bottle (25 mL)	2 bottles (125 mL/bottle)
Component C: Limulus Amebocyte Lysate	Freeze (< -15 °C), Minimize light exposure	1 vial	2 vials
Component D: E.coli Endotoxin Standard	Desiccated, Freeze (< -15 °C), Minimize light exposure	1 vial (100 EU/mL)	2 vials (100 EU/mL)
Component E: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)	1 vial (600 µL)

OVERVIEW

Lipopolysaccharide (LPS), also known as endotoxin, is the major component of the outer membranes of Gram-negative bacteria. LPS is a potent stimulator of the vertebrate innate immune system and can cause fever, septic shock and eventually death. It is also recognized as a biomarker for the detection of bacterial pathogen invasion, and responsible for the development of inflammatory response and endotoxic shock in extreme cases. Detection of LPS in biological materials, such as protein, peptide or antibody sample, is a critical task for biomanufacturing and bioprocessing. Portelite™ Rapid Fluorimetric Endotoxin Detection Kit uses Endotoxin Green™, a sensitive fluorogenic substrate. Endotoxin Green™ can be hydrolyzed in the presence of endotoxins and the Limulus Amebocyte Lysate (LAL), an extract of blood cells from a horseshoe crab, to generate strong green fluorescence. The endotoxin activity is proportional to the fluorescence intensity resulted from the hydrolysis of Endotoxin Green™. The kit is optimized for Cytocite™ and Qubit™ fluorometers, and can detect a broad range of endotoxin (from 1 EU/ml to 0.002EU/ml) present in the sample.

AT A GLANCE

Protocol summary

1. Prepare Limulus Amebocyte Lysate (LAL) working solution
2. Add E.coli Endotoxin Standards and test samples (50 µL)
3. Add Limulus Amebocyte Lysate working solution (50 µL)
4. Incubate at 37 °C for 30 minutes
5. Prepare and add Endotoxin Green™ working solution (100 µL)
6. Monitor fluorescence with CytoCite™ Fluorometer within 10 minutes

Important

Thaw all the kit components at room temperature before starting the experiment.

All Materials used in the experiment should be endotoxin-free, such as: disposable tubes or 1.5 mL microcentrifuge tubes, disposable pipette tips, and tubes. The cleanliness of all labware is required to accurately detect levels of endotoxin in a given sample.

KEY PARAMETERS

CytoCite Fluorometer

Emission	510-580 nm
Excitation	480 nm
Instrument specification(s)	0.2 mL, thin-wall PCR tube

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Endotoxin Green™ stock solution

For **kit# 60008**, add 50 µL of DMSO into one vial of Endotoxin Green™ (Component A) to make Endotoxin Substrate stock solution. For **kit# 60009**, add 500 µL of DMSO into one vial of Endotoxin Green™ (Component A) to make Endotoxin Substrate stock solution.
Note Keep from light.

Limulus Amebocyte Lysate stock solution

For **kit# 60008**, add 500 µL Endotoxin-Free Water (Component B) to the vial of Limulus Amebocyte Lysate (Component C) to make 5X Limulus Amebocyte Lysate (LAL) stock solution. For **kit# 60009**, add 2.5 mL Endotoxin-Free Water (Component B) to the vial of Limulus Amebocyte Lysate (Component C) to make 5X Limulus Amebocyte Lysate (LAL) stock solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/60008

E.coli Endotoxin Standard solution

Add 10 µL of 100 EU/mL E.coli Endotoxin Standard solution to 990 µL of Endotoxin-Free Water (Component B) to generate 1 EU/mL E.coli Endotoxin standard solution. Then take 1 EU/mL E.coli Endotoxin Standard solution, and perform 1:3 serial dilutions in Endotoxin-Free Water (Component B) to get serially diluted E.coli Endotoxin Standards 1 to 0.001 EU/mL.

PREPARATION OF WORKING SOLUTION

Endotoxin Green™ working solution

For **Kit# 60008**, add 50 µL of Endotoxin Green™ stock solution into 5

mL of Endotoxin-Free Water (Component B) to make a total volume of 5.05 mL Endotoxin Green™ working solution. For **Kit# 60009**, add 500 µL of Endotoxin Green™ stock solution into 50 mL of Endotoxin-Free Water (Component B) to make a total volume of 50.5 mL Endotoxin Green™ working solution.

Note Each test needs 100 µL, please prepare the amount of Endotoxin Green™ working solution as needed and before use. Please also keep the working solution from light after preparation, and use Endotoxin-Free bottle or tube and store unused stock solutions at -20 °C.

Limulus Amebocyte Lysate (LAL) working solution

For **kit# 60008**, add 500 µL of Limulus Amebocyte Lysate (LAL) stock Solution into 2 mL of Endotoxin-Free Water (Component B) to make a total volume of 2.5 mL Limulus Amebocyte Lysate (LAL) working solution. For **kit# 60009**, add 2.5 mL of Limulus Amebocyte Lysate (LAL) stock Solution into 10 mL of Endotoxin-Free Water (Component B) to make a total volume of 12.5 mL Limulus Amebocyte Lysate (LAL) working solution.

Note Each test needs 50 uL, please prepare the amount of LAL working solution as needed and before use. Please also keep the working solution from light after preparation, use Endotoxin-Free bottle or tube, and store unused stock solutions at -20 °C.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare E.coli Endotoxin Standards, blank controls or test samples (50 µL) in a 0.2 mL PCR tube (Cat# CCT100).
2. Add 50 µL of Limulus Amebocyte Lysate working solution to each tube of E.coli Endotoxin Standard, blank control and test samples.
3. Mix well and incubate for 30 minutes at 37 °C.
4. Add 100 µL of Endotoxin Green™ working solution to each tube of Endotoxin Standard, blank control, and test samples to make the total assay volume 200 µL/well.
5. Insert the samples into CytoCite™ and monitor the fluorescence with green fluorescent channel. Follow the procedure appropriate for CytoCite™ Fluorometer. See the link below for detailed instructions: https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer.

   Note For best results, read between 2 to 10 minutes after adding the working solution.

   Note 50 µL of 25% acetic acid can be added to stop the reaction.

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

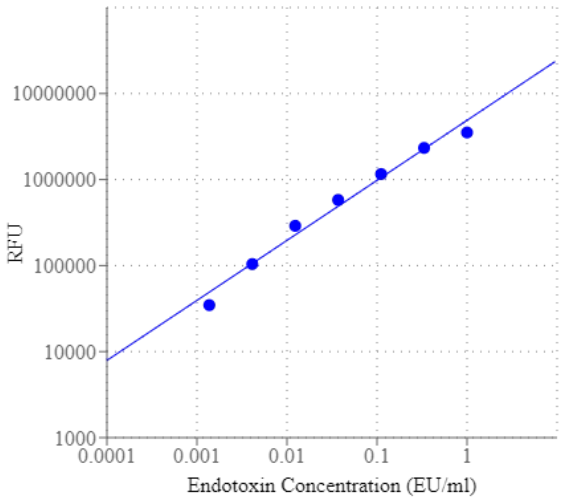

Figure 1. E.coli Endotoxin dose response was measured in a 0.2 mL, thin-wall PCR tube using CytoCite™ with Green fluorescent channel. As low as 0.002 EU/mL of E.coli Endotoxin can be detected with 10 minutes incubation.

DISCLAIMER

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