Amplite™ Fluorimetric Endotoxin Detection Kit

Catalog number: 60006
Unit size: 100 Tests

Component	Storage	Amount
Component A: Endotoxin Green™	Desiccated, Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Endotoxin-Free Water	Freeze (< -15 °C)	1 bottle (25 mL)
Component C: Limulus Amebocyte Lysate	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: E.coli Endotoxin Standard	Desiccated, Freeze (< -15 °C), Minimize light exposure	1 vial (100 EU/mL)
Component E: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Lipopolysaccharide (LPS), also known as endotoxin, is the major component of the outer membrane of Gram-negative bacteria. LPS is a potent stimulator of the vertebrate innate immune system and can cause fever, septic shock and eventually death. LPS is also recognized as a biomarker for the detection of bacterial pathogen invasion, and is 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 in biological manufacturing and processing. Amplite™ Fluorimetric Endotoxin Detection Kit uses Endotoxin Green™, a sensitive fluorogenic substrate. Endotoxin Green™ is hydrolyzed in the presence of endotoxins and the Limulus Amebocyte Lysate (LAL), an extract of blood cells from a horseshoe crab. The hydrolyzed product of Endotoxin Green™ generates strong green fluorescence. The endotoxin activity is proportional to the fluorescence intensity resulted from the hydrolysis of Endotoxin Green™. Amplite™ Fluorimetric Endotoxin Detection Kit can detect a broad range of endotoxin (from 1 EU/ml to 0.001 EU/ml). It is very sensitive and can detect as low as 0.001 EU/mL of endotoxin within 30 min.

AT A GLANCE

Protocol summary

1. Prepare Endotoxin Green™ working solution
2. Add E.coli Endotoxin Standards and test samples (25 µL)
3. Add Limulus Amebocyte Lystate solution (25 µL)
4. Incubate at 37 °C for 30 minutes
5. Add Endotoxin Green™ working solution (50 µL)
6. Read fluorescence intensity at Ex/Em=490/525 nm 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 disposable 96-well microplates or plate strips. The cleanliness of all labware is required to accurately detect levels of endotoxin in a given sample.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black
Instrument specification(s)	Top read mode

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.

1. 100 X Endotoxin Green™ stock solution
Add 50 µL of DMSO into the vial of Endotoxin Green™ (Component A) to make 100X Endotoxin Green™ stock solution.

Note Keep from light.

2. 5X Limulus Amebocyte Lysate solution
Add 500 µL of Endotoxin-Free Water (Component B) into the vial of Limulus Amebocyte Lysate (Component C) to make 5X Limulus Amebocyte Lysate solution.

PREPARATION OF STANDARD SOLUTION

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

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 (ES1). Then take 1 EU/mL E.coli Endotoxin Standard solution (ES1) and perform 1:3 serial dilutions in Endotoxin-Free Water (Component B) to get serially diluted E.coli Endotoxin Standards (ES2 - ES7).

PREPARATION OF WORKING SOLUTION

1. Endotoxin Green™ working solution
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.

Note Prepare the amount of Endotoxin Green™ working solution as needed. Keep the working solution from light.

2. Limulus Amebocyte Lysate (LAL) working solution
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.

Note Prepare the amount of LAL working solution as needed and before use. Using the Endotoxin-Free bottle or tube.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of E.coli Endotoxin Standards and test samples in a solid black 96-well microplate. ES=E.coli Endotoxin standards (ES1-ES7, 1.00 to 0.001 EU/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
ES1	ES1	...	...
ES2	ES2	...	...
ES3	ES3		
ES4	ES4		
ES5	ES5		
ES6	ES6		
ES7	ES7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
ES1-ES7	25 µL	Serial dilutions (1.00-0.001 EU/mL)
BL	25 µL	Endotoxin-Free Water

TS	25 µL	Test Samples

1. Prepare E.coli Endotoxin Standards (ES), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 12.5 µL of reagent per well instead of 25 µL.

2. Add 25 µL of 2X Limulus Amebocyte Lysate solution to each well of E.coli Endotoxin Standard, blank control and test samples.

3. Mix well and incubate for 30 minutes at 37 °C.

4. Add 50 µL of Endotoxin Green™ working solution to each well of E.coli Endotoxin Standard, blank control, and test samples to make the total assay volume 100 µL/well. For a 384-well plate, add 25 µL of Amplite™ Endotoxin Green working solution into each well instead, for a total volume of 50 µL/well.

5. Monitor the fluorescence intensity at Ex/Em=490/525 nm, cutoff 515 nm.

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

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

EXAMPLE DATA ANALYSIS AND FIGURES

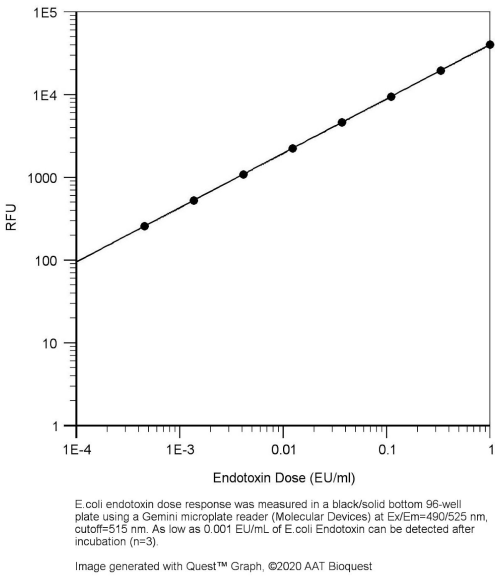

Figure 1. E.coli endotoxin dose response was measured in a black/solid bottom 96-well plate using a Gemini microplate reader (Molecular Devices) at Ex/Em=490/525 nm, cutoff=515 nm. As low as 0.001 EU/mL of E.coli Endotoxin can be detected after incubation (n=3).

DISCLAIMER

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