Amplite® Colorimetric Endotoxin Detection Kit

Catalog number: 60007
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 60007)
Component A: Endotoxin Yellow™	Freeze (< -15 °C), Minimize light exposure, Desiccated	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	Freeze (< -15 °C), Minimize light exposure, Desiccated	1 Vial (100 EU/mL)
Component E: DMSO	Refrigerated (2-8 °C)	1 vial

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 in biomanufacturing and bioprocessing. Amplite®™ Colorimetric Endotoxin Detection Kit uses Endotoxin Yellow™, a sensitive chromogenic substrate. Endotoxin Yellow™ 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 an intense yellow colored product. The endotoxin activity is proportional to the absorbance of the yellow product resulted from the hydrolysis of Endotoxin Yellow™. Amplite®™ Colorimetric Endotoxin Detection Kit can detect a broad range of endotoxin from 1 EU/ml to 0.002 EU/ml.

AT A GLANCE

Protocol summary

1. Prepare Endotoxin Yellow™ 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 Yellow™ working solution (50 µL)
6. Read optical density at 498 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

Absorbance microplate reader

Absorbance	498 nm
Recommended plate	Clear bottom

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

100X Endotoxin Yellow™ stock solution

Add 50 µL of DMSO into the vial of Endotoxin Yellow™ (Component A) to make 100X Endotoxin Yellow™ stock solution.
Note Keep from light.

Limulus Amebocyte Lysate (LAL) Stock Solution

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.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/60007

E.coli Endotoxin Standard solution

Add 40 µL of 100 EU/mL E.coli Endotoxin Standard solution to 360 µL of Endotoxin-Free Water (Component B) to generate 10 EU/mL E.coli Endotoxin Standard solution (ES1). Then take 10 EU/mL E.coli endotoxin standard solution (ES1) and perform 1:2 serial dilutions in Endotoxin-Free Water (Component B) to get serially diluted E.coli Endotoxin Standards (ES2 - ES7).

PREPARATION OF WORKING SOLUTION

Endotoxin Yellow™ working solution

Add 50 µL of Endotoxin Yellow™ stock solution into 5 mL of Endotoxin-Free Water (Component B) to make a total volume of 5.05 mL Endotoxin Yellow™ working solution.
Note Prepare the amount of endotoxin substrate working solution as needed. Keep the working solution from light.

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 clear bottom 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 to 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 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 Yellow™ 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 Endotoxin Yellow™ solution into each well instead, for a total volume of 50 µL/well.
5. Monitor the optical density at 498 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

Placeholder for image details

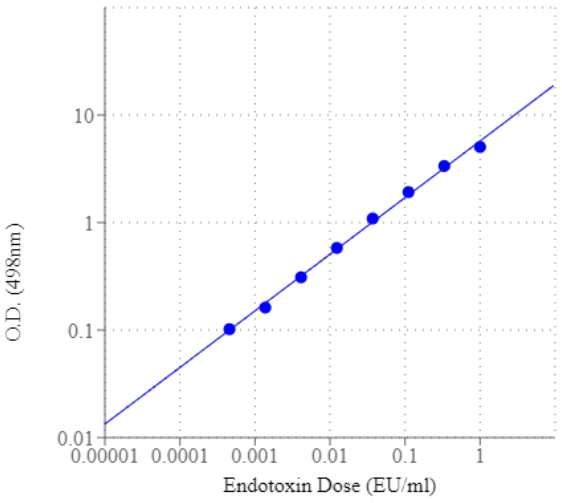

Figure 1. E.coli Endotoxin dose response was measured in an all-clear 96-well plate using a with a Spectrum Max microplate reader (Molecular Devices) at OD 498nm. As low as 0.001 EU/mL of E.coli Endotoxin can be detected with 10 minutes incubation (n=3).

DISCLAIMER

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