ab324126 – Endotoxin Detection Kit (Fluorimetric)

For the detection of a broad range of endotoxin (from 1 EU/ml to 0.001 EU/ml) using Endotoxin Green™, a sensitive fluorogenic substrate.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324126

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Endotoxin Green™	1 vial	-20°C (Store in the dark)
Endotoxin-Free Water	1 bottle (25 mL)	-20°C
Limulus Amebocyte Lysate	1 vial	-20°C (Store in the dark)
E.coli Endotoxin Standard	1 vial (100 EU/mL)	-20°C (Store in the dark)
DMSO	1 vial (100 µL)	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Fluorescence microplate reader (515/525 nm) – Top read mode
- Solid black plates

Protocol Summary

1. Prepare Endotoxin Green™ working solution.
2. Add E.coli Endotoxin Standards and test samples (25 µL).
3. Add Limulus Amebocyte Lysate 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 and centrifuge the vials briefly before opening.

Note: *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.*

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.

100 X Endotoxin Green™ stock solution

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

Note *: Keep from light.*

5X Limulus Amebocyte Lysate solution

1. Add 500 µL of Endotoxin-Free Water into the vial of Limulus Amebocyte Lysate to make 5X Limulus Amebocyte Lysate solution.

Preparation of Standard Solution

E.coli Endotoxin Standard solution

1. Add 10 µL of 100 EU/mL E.coli Endotoxin Standard solution to 990 µL of Endotoxin-Free Water 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 to get serially diluted E.coli Endotoxin Standards (ES2 - ES7).

Preparation of Working Solutions

Endotoxin Green™ working solution

1. Add 50 µL of Endotoxin Green™ stock solution into 5 mL of Endotoxin-Free Water 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.*

Limulus Amebocyte Lysate (LAL) working solution

1. Add 500 µL of Limulus Amebocyte Lysate (LAL) Stock Solution into 2 mL of Endotoxin-Free Water 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.*

Experimental Protocol

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

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.

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 Sample

Table 2. Reagent composition for each well of a 96-well microplate.

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 1X 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 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.*

For technical support contact information, visit: www.abcam.com/contactus

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