EndotoxinSensor™ Chromogenic LAL Endotoxin Assay

(Cat # 786-2441)

For research use only

INTRODUCTION

EndotoxinSensor™ Chromogenic LAL Endotoxin Assay is an in vitro end-point endotoxin test for Research Use Only (RUO), not for the detection of endotoxin in a licensed reagent, clinical samples or the diagnosis of human diseases. A measurable endotoxin concentration range from 0.01 to 1.0 EU/mL can be achieved. Lyophilized Amebocyte Lysate (LAL) reagent is derived from amebocyte lysate from the horseshoe crab (*Tachypleus tridentatus*).

APPLICATION

• Simple and sensitive for the detection of the presence of Lipopolysaccharides in samples

FEATURES

• Ready to use reagents and materials
• High specificity and high sensitivity
• Minimum handling time

STORAGE:

The kit is shipped with blue ice. Store components according to the box labels, protect from the light. Do not freeze the kit or any of its components

ITEMS SUPPLIED

Description	Size	QTY
LAL	-	2
E. coli Endotoxin Standard	-	2
Chromogenic Substrate	-	2
LAL Reagent Water	50ml	3
Color - stabilizer #1	-	2
Color - stabilizer #2	-	2
Color - stabilizer #3	-	2
Endotoxin - free Vials	5/pk	10
Endotoxin - free Tips, 200 µl	96/pk	1
Endotoxin - free Tips, 1 ml	6/bag	2
Incubation Rack	1	1

ADDITIONAL ITEMS REQUIRED

- Water bath or heat block
- Concentrated Hydrochloric Acid (36-38%)
- Spectrophotometer with a 545 nm filter

PREPARATION BEFORE USE

LAL:

Reconstitute LAL by adding 1.7ml LAL reagent water. Gently mix by inverting. Avoid foaming during the process.

Note: Always make fresh before use.

Chromogenic Substrate:

Add 1.7ml LAL Reagent Water to reconstitute the Chromogenic Substrate. Store at 2-8°C (stable up to 1 month) and protect from the light.

Color Stabilizer 1:

Add 2ml concentrated HCL (36-38%) to 50mL LAL Reagent Water to make a concentration of 0.46M HCL, mix well.

Add 10ml of 0.46M HCL to reconstitute Color Stabilizer 1. Stable up to 1 week at 2-8°C.

Note: Concentrated HCL is very dangerous. This step should be carried out in a hood with proper precaution.

Color Stabilizer 2:

Add 10ml of LAL reagent water to reconstitute Color Stabilizer 2. Stable up to 1 week at 2-8°C.

Color Stabilizer 3:

Add 10ml of LAL reagent water to reconstitute Color Stabilizer 3. Stable up to 1 week at 2-8°C.

PROTOCOL:

PREPARATION OF ENDOTOXIN STANDARDS

For minimizing interference, it is important to prepare the appropriate diluted endotoxin standard in the same diluent used for the test endotoxin sample. Prepare a 1 EU/ml endotoxin standard stock solution by adding 15ml LAL reagent water. In each assay, at least 4 endotoxin standard solutions need to be prepared to cover the desired concentration range to prepare a standard curve. If the endotoxin concentration for the test sample is expected to be in the range of 0.1– 1.0 EU/ml, the diluted endotoxin standard should be as below:

For 0.1 – 1.0 EU/ml standard:

1.0 EU/ml Endotoxin Standard (µl)	LAL reagent water (µl)	Final standard concentration (EU/ml)
400	0	1.0
200	200	0.5
100	300	0.25
40	360	0.1
0	400	0

If the endotoxin concentration for the test sample is expected to be in the range of 0.01– 0.1 EU/ml, the diluted endotoxin standard should be as below:

For 0.01 – 0.1 EU/ml standard:

0.1 EU/ml Endotoxin Standard (µl)	LAL reagent water (µl)	Final standard concentration (EU/ml)
400	0	0.1
200	200	0.05
100	300	0.025
40	360	0.01
0	400	0

PROCEDURES

1. Add 100 µl of standards, samples and LAL reagent water into different endotoxin free vials and label them.
2. Add 100 µl of reconstituted LAL to each vial and mix well. Avoid foaming.
3. If the expected endotoxin level of the target samples is between 0.01 – 0.1 EU/ml, incubate all the vials at 37°C for T1. If the expected endotoxin level of the target samples is between 0.1 – 1.0 EU/ml, incubate all the vials at 37°C for T2. The optimum value of T1 and T2 is referred to the label on the box.
4. Add 100 µl of reconstituted chromogenic substrate solution to each vial. Mix gently, avoid foaming.



5. Incubate all the vials at 37°C for 5 minutes.
6. Add 500 µl of color stabilizer 1. Mix well, avoid foaming.
7. Add 500 µl of color stabilizer 2. Mix well, avoid foaming.
8. Finally add 500 µl of color stabilizer 3. Mix well, avoid foaming.
9. Measure absorbance at 545 nm. Use distilled water as a blank to set autozero. In case of using microplate reader, transfer 200 µl of final solution into a 96-well plate and take the absorbance at 545 nm

CALCULATION OF CONCENTRATION

1. Subtract the blank absorbance (LAL reagent water blank) from each vial reading.
2. To generate a standard curve, plot the blank-subtracted absorbance at Y-axis and corresponding endotoxin concentration at X-axis.
3. Draw a best-fit straight line and calculate the endotoxin concentration of each sample from the graphically generated equation.

TROUBLESHOOTING

Problem	Possible cause	Recommendations
Higher background absorbance	Reagents may be contaminated	• Always use endotoxin-free materials • Wear disposable gloves • Make sure working area is endotoxin-free
Standard curve is not linear	Insufficient endotoxin mixing	• Add enough LAL reagent water to dissolve endotoxin standard completely • Place it on a vortex mixer for 15 minutes • Strictly follow the protocol specially the incubation time