



VDL 612.2 ENDOTOXIN ASSAY: ASSAY FOR LIMULUS AMEBOCYTE LYSATE

1. Purpose

- 1.1. The purpose of this protocol is test purified viral vectors for endotoxin contamination.
- 1.2. This procedure is routinely performed in the Vector Development Laboratory (VDL) following Good Laboratory Practices (GLP).

2. Abbreviations and Definitions

2.1.	SOP	Standard Operating Procedure
2.2.	VDL	Vector Development Laboratory
2.3.	GLP	Good Laboratory Practice
2.4.	LAL	Limulus Amebocyte Lysate
2.5.	SDS	Sodium dodecylsulfate
2.6.	Stop Reagent	10% SDS

3. Equipment, Materials, and Reagents

NOTE: All materials in contact with cells must be sterile, pyrogen-free and used according to the manufacturer's directions unless stated otherwise. Equivalent materials and equipment may be used but all changes must be recorded.

3.1. Equipment

- 3.1.1. Heat block at 37°C
- 3.1.2. Stopwatch
- 3.1.3. Vortex mixer
- 3.1.4. Microplate reader
- 3.1.5. Microplate adapter for heater

3.2. Materials

3.2.1.	Disposable endotoxin-free glass dilution tubes (13x100 mm)	Lonza
3.2.2.	96- Microplates	Corning
3.2.3.	Serological pipets	Corning
3.2.4.	Sterile pipet tips	VWR

3.3. Reagents

3.3.1.	LAL Assay Kit	Lonza
3.3.2.	SDS	Sigma
3.3.3.	Stop reagent	VDL
3.3.4.	NaOH	Sigma





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3.3.5.	0.1N NaOH in LAL Reagent Water	VDL
3.3.6.	HCI	Sigma
3.3.7.	0.1 N HCl in LAL Reagent Water	VDL

3.4. Starting Materials

3.4.1. 50 µL test sample

3.5. **Test Sample Identification**

- 3.5.1. The bar code on the plasmid will be scanned and compared to the computer database to ensure the correct sample is being processed.
- 3.5.2. One copy of the barcode will be printed and applied to a copy of this SOP for the final record.

4. Procedure

Sample collection and preparation

- 4.1. Samples to be tested must be stored in such a way that all bacteriological activity is stopped or the endotoxin level may increase with time.
 - For example, store samples at 2-8°C for less than 24 hours; samples stored longer than 24 hours should be frozen.
- 4.2. If the container of diluent used to rehydrate the reagents has been opened previously, the diluent alone must be tested for endotoxin contamination.

Preparation of Standards

- 4.3. In each series of determinations, four standard endotoxin solutions are be used.
- 4.4. Prepare a solution containing 1.0 EU/mL endotoxin by diluting 0.1 mL of the endotoxin stock solution with (X-1)/10 mL of LAL Reagent Water in a 15 mL conical tube, where X equals the endotoxin concentration of the vial.
 - For example, if X = 23 EU/mL, then dilute 0.1 mL of the endotoxin stock solution with 2.2 ml, (23-1)/10, LAL Reagent Water.
- 4.5. Vortex solution vigorously for at least 1 minute before proceeding.
- 4.6. Transfer 0.5 mL of this 1.0 EU/ mL solution into 0.5 mL of LAL Reagent Water in a suitable container and label 0.5 EU/mL.
- 4.7. Vortex this solution vigorously for at least 1 minute before use.
- 4.8. Transfer 0.5 mL of the 1.0 EU/mL solution into 1.5 mL of LAL Reagent Water in a suitable container and label 0.25 EU/mL.
- 4.9. Vortex this solution vigorously for at least 1 minute before use.
- 4.10. Transfer 0.1 mL of the 1.0 EU/mL solution into 0.9 ml of LAL Reagent Water in a





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suitable container and label 0.1 EU/mL.

4.11. Vortex this solution vigorously for at least 1 minute prior to use.

Microtiter Plate Protocol

- 4.12. Pre-equilibrate the microplate at 37°C in the heating block adapter.
- 4.13. While leaving the microplate at 37°C, carefully dispense 50 μL of sample or standard into the appropriate microplate well.
- 4.14. Each series of determinations must include a blank plus the four endotoxin standards run in duplicate. The blank wells contain 50 μL of LAL Reagent Water instead of sample.
 Note: All reagent additions and incubation times must be identical.
- 4.15. At time T = 0, add 50 μ L of LAL to the first microplate well. Begin timing as the LAL is added.

Note: It is important to be consistent in the order of reagent addition from well to well or row to row, and in the rate of pipetting.

- 4.16. Once the LAL has been dispensed into all microplate wells containing samples or standards, briefly remove the microplate from the heating block adapter and repeatedly tap the side of the plate to facilitate mixing. Return the plate to the heating block adapter and replace cover.
- 4.17. At T = 10 minutes, add 100 μ L of substrate solution (prewarmed to 37°C).
- 4.18. Pipette the substrate solution in the same manner as in Step 4.18. Maintain a consistent pipetting rate. Once the substrate solution has been dispensed into all microplate wells, briefly remove the microplate from the heating block adapter and repeatedly tap the side of the plate to facilitate mixing. Return the plate to the heating block adapter and replace cover.
- 4.19. At T = 16 minutes, add 100 μ L of stop reagent. Maintain the same pipetting order as in Steps 4.15 and 4.17. Once the stop reagent has been dispensed into all microplate wells, remove the plate and repeatedly tap the side of the plate.
- 4.20. Read the absorbance of each microplate well at 405-410 nm using distilled water to adjust the photometer to zero absorbance.
- 4.21. NOTE: The performance characteristics of certain microplate readers are optimal with sample volumes less than 300 μ L. The final reaction volume per well can be reduced by adding only 50 μ L of the above suggested stop reagents without adversely affecting the test results.

Calculations





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Note: The follow calculation can be performed in a statistical program a simple as Microsoft Excel.

Note: Under the standard conditions, the absorbance at 405-410nm is linear in the concentration range 0.1 to 1.0. EU/mL endotoxin.

- 4.22. Subtract the mean absorbance of the blank from the mean absorbance value of the standards and sample to calculate mean Δ absorbances.
- 4.23. Plot the mean Δ absorbance for the four standards on the y-axis vs. the corresponding endotoxin concentration in EU/mL on the x-axis.
- 4.24. Draw a best fit straight line between these points and determine endotoxin concentration of samples graphically.

5. Data Collection and Management

- 5.1. All data will be collected in a laboratory notebook. The data will be entered into the notebook in the form of a print out of raw data from the photometer reader program. A computerized copy of the raw data will also be stored on the VDL server.
- 5.2. All calculations of endotoxin level and associated graphs will be written out in the laboratory notebook.
- 5.3. Deviations of the protocol will be recorded in the laboratory notebook and on the Lab Meeting Sheet.

6. Review and Revisions Written by: Director, VDL Reviewed by: Director, QA/QC Reviewed by: Director, Vector Production Date Issued: 1/30/2006 Replaces VDL 612.0 Annual Review:

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2012	
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