

ENDOTOXIN INDICATORS

INTENDED USE:

Endotoxin Indicators are designed for validation or monitoring depyrogenation processes such as dry-heat sterilization and washing procedures. The extent of depyrogenation may be measured by comparing the levels of endotoxin before and after a depyrogenation cycle using Charles River Endosafe® LAL Reagent. The U. S. Pharmacopeia suggests that a depyrogenation cycle should reduce an Endotoxin Indicator by at least 1000 fold (3-log reduction) in endotoxic activity as measured by LAL (Limulus Amebocyte Lysate) methods.¹

EXPLANATION OF TEST:

Dry-heat sterilization is the preferred method for rendering glassware and other heat-stable materials free of endotoxin. The U. S. Pharmacopeia, the Parenteral Drug Association's Technical Bulletin No. 7, the LAL Users Group and others have described approaches for preparation and use of Endotoxin Indicators.¹⁻⁶

Charles River Endosafe®'s Endotoxin Indicators have been carefully prepared and assayed for endotoxin content so that the endotoxicity in the vials may be recovered within a two-fold dilution of the labeled potency of the reference standard lysate. The magnitude of depyrogenation may be assessed by comparing the amount of endotoxin in exposed or processed Indicators to the amount in unmodified Indicators. The most convenient method for Indicator quantitation is to select a kinetic LAL method and use either Charles River Endosafe® KTA (Product Code R150), a kinetic turbidimetric assay reagent, or Charles River Endosafe® Endochrome-K (Product Code R170), a kinetic chromogenic assay reagent.

COMPOSITION:

Each vial contains approximately 10 Million EU (Endotoxin Units) of lipopolysaccharide from *E.coli* 055:B5, as certified by Charles River Endosafe® with reference materials. The Indicator vial, made of Type I borosilicate glass, appears empty because the air-dried endotoxin is not mixed with fillers or stabilizers.

Note: The biological activity for labeling of the Endotoxin Indicators was determined using the Gel-clot reference lysate. However, an RSE/CSE analysis has not been performed with individual LAL batches. Prior to use, the end user may wish to either verify the sensitivity of the Endotoxin Indicators to be within a two-fold of label claim, or perform testing to assign a different biological activity with a specific LAL batch.

STORAGE: Store vials at 2 to 25 degrees C with closures and stoppers intact.

WARNING: Endotoxin Indicators contain pyrogenic amounts of endotoxin should they be administered by parenteral routes. Endotoxin Indicators are for in-vitro use only, and not to be used in humans or animals.

PROCEDURES:

Preparation of Endotoxin Indicators: The 10 Million EU Indicators may be used to make Endotoxin Indicators of lesser potency in the glassware of your choice. Rehydrate with sufficient LAL Reagent Water to permit dispensing 0.1 mL aliquots of the desired potency.

Exposure Procedure. For oven validation, expose Endotoxin Indicators at the sites specified. Immediately before oven exposure, remove the vial closure and stopper and replace with aluminum foil and expose the Indicator vial to the depyrogenation process.

Note: Vials labels are heat resistant and do not need to be removed prior to depyrogenation.

Preparation for Analysis. After the Endotoxin Indicators have been baked or processed, the vials from the depyrogenation process and one or two unexposed Indicators (positive controls) should be prepared for analysis. The Indicators should be assayed by a validated LAL-test procedure whereby the reagent sensitivity is verified by a certified CSE (Control Standard Endotoxin, Product Code E110 or E120) or RSE. All vials should be prepared for LAL testing by rehydrating each Indicator vial with 1 mL of LAL Reagent Water and vortexing for two minutes initially, and then for one minute every ten minutes for one-half hour. The LAL analysis should begin promptly for best recovery.

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Assay Procedure by Kinetic LAL Methods. A convenient method for analysis of Endotoxin Indicators is the use of kinetic LAL methods from Charles River Endosafe®. The analysis is conducted by setting up a properly controlled kinetic chromogenic or turbidimetric analysis using a 2-log standard curve of 0.05 to 5 EU/mL, and testing a dilution of the positive control and undiluted samples of the baked or exposed vials. Both the positive control(s) (PPC) and exposed vials should have PPC(s) run to show absence at interference. Percent recovery for the PPC should be within 50% - 200% to be valid. The results of the analysis more than satisfies the conditions for >3-log cycle reduction of endotoxin if $\log (\text{EU/vial of control vial}) - \log (\text{EU/vial of exposed vial})$ is >3.

An example calculation is provided wherein 10,000,000 EU per vial was recovered in the positive controls and endotoxin levels recovered in exposed vials were less than lambda, the lowest point on the standard curve:

$$\begin{aligned}\text{log reduction} &= \log 10,000,000 \text{ EU/mL} - \log 0.05 \text{ EU/mL} \\ &= 7 - (-1.301) \\ &= 8.3\end{aligned}$$

REFERENCES:

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2. Parenteral Drug Association, Inc., Depyrogenation, Technical Report No. 7, 1985.
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4. Weary, M and F Pearson. A manufacturer's guide to depyrogenation. Biopharm. 1, 1988.
5. Nakata, T, Destruction of challenged endotoxin in a dry heat oven, PDA J. Pharm. Sci. Technol., 48:59, 1994.
6. Hecker, W, D Witthauer and A Staerk. Validation of dry heat inactivation of bacterial endotoxins, PDA J. Pharm. Sci. Technol., 48:197, 1994.

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