

Depyrogenation Validation Endotoxin Challenge Technical Tip

Suggested procedure to
make your own
endotoxin challenge vials

By Lonza Scientific Support, U.S.

Goal:

To demonstrate greater than a 3-log reduction in endotoxin concentration after depyrogenation.

For this purpose, Endotoxin *E. coli* O55:B5 (Lonza Catalog No. [00193783](#)) might be used. The following procedure is a suggestion for how to prepare spiked material for depyrogenation studies.

The example calculations below assume the potency of the endotoxin is at the lower limit of 1.25×10^6 EU/vial.*

1. Reconstitute the vial with 5 mL of LAL Reagent Water (LRW, for example Lonza Catalog No. W50-100) to yield 250,000 EU/mL and vortex for 30 min at >1,250 RPM.
2. Dilute the stock endotoxin 1/5 with LRW to yield 50,000 EU/mL. (The dilution of the stock can be adjusted to achieve the desired yield.)
3. Spike the material (typically a 2 mL glass vial) with 0.1 mL of the diluted stock endotoxin to result in a spike of 5,000 EU/vial.

Note: The endotoxin indicator should be the same as or similar in composition to the surface being depyrogenated.



4. Air dry or lyophilize the vials.
5. Bake or wash test vials and save at least 3 unbaked/unwashed positive control vials.
6. Reconstitute each baked/washed vial and unbaked/unwashed positive control vial with 1 mL of LRW.
7. Vortex each vial for 30 minutes at >1,250 RPM.
8. Test baked/washed vials undiluted. Dilute positive control vials 1/10,000.

*The potency for Lonza Catalog No. **00193783** kits may vary from lot to lot. Be sure to use the potency value indicated on your lot's Certificate of Analysis for calculations or establish the value by testing.

Results:

1. Unbaked/unwashed positive control vials: Expect 50 – 80% recovery of the spike.
2. Baked/washed vials: Endotoxin recovery should show a minimum 3-log reduction of the EU/mL concentration of the positive control vials if the depyrogenation method was successful.

Example: The endotoxin concentration of the positive control is established to be 3,500 EU/vial after testing. A 3-log reduction from this value is 3,500 EU/vial ÷ 1,000 or 3.5 EU/vial. So, to confirm a successful depyrogenation cycle, the endotoxin recovery result for the baked/washed vials must be less than 3.5 EU/vial.

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