

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* 055: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.



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 $3,500 \text{ EU/vial} \div 1,000$ 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.

*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.

www.lonza.com/pharmabiotech

www.lonza.com/endo

Contact Information

North America

Customer Service: 800 638 8174 (toll free)

order.us@lonza.com

Scientific Support: 800 521 0390 (toll free)

scientific.support@lonza.com

Europe

Customer Service: +32 87 321 611

order.europe@lonza.com

Scientific Support: +32 87 321 611

scientific.support.eu@lonza.com

International

Contact your local Lonza distributor

Customer Service: +1 301 898 7025

Fax: +1 301 845 8291

scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

Unless otherwise noted, all trademarks herein are marks of the Lonza Group Ltd or its affiliates. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights.

© Copyright 2013, Lonza Walkersville, Inc. All rights reserved.
TS-DepyVal 07/13 RT-DS010
