

## ENDOTOXIN-SPECIFIC BUFFER SOLUTION (CARBOXYMETHYLATED CURDLAN)



Catalog #BG120

### INTENDED USE:

Endotoxin-Specific (ES) Buffer Solution is an adjunct for endotoxin testing by Limulus Amebocyte Lysate (LAL) methods. Its intended use is to block the interference of  $\beta$ -D-Glucans and LAL Reactive Material (LAL-RM) during LAL testing.<sup>1</sup> The USFDA Guideline for LAL test applications allows the use of adjuncts to overcome interfering LAL-test conditions.<sup>3</sup>

### EXPLANATION OF TEST:

The LAL test is the most sensitive and specific means to detect and measure bacterial endotoxin. The LAL reaction is an enzyme-mediated process which requires a neutral pH environment and a proper balance of monovalent and divalent cations.<sup>2,3</sup> The LAL test was considered specific for endotoxin until a  $\beta$ -D-Glucan, known as LAL-RM, was reported to activate LAL through an alternate enzymatic pathway.<sup>4,5</sup> Common sources of this glucose polymer are yeast cell wall and cellulosic sources such as hollow fiber membranes. The confusing part of this specificity problem is that commercially available LAL Reagents vary greatly in reactivity to LAL-RM.<sup>2,6</sup> The consequences of this variability among reagents to LAL-RM interference are a loss of confidence in specificity and a lack of inter-laboratory agreement on LAL-test results.

The chronic effects of Glucans in mammalian systems are not fully defined, but patients undergoing renal dialysis are exposed to LAL-RM without apparent serious adverse effect.<sup>2,6</sup> It is prudent to use a Glucan-sensitive LAL Reagent so sources of false-positive LAL-test results can be identified and eliminated, or causes of conflicting laboratory data can be explained. If LAL-RM interference is suspected, materials may be tested with and without a Glucan blocker to determine whether or not Glucans are present. On the other hand, when a material has a history of producing results consistent with traces of LAL-RM, the Glucan blocker may be used with the LAL Reagent to avoid this non-specificity problem.<sup>7,8</sup> It is advantageous to use kinetic LAL methods when Glucans are suspected because endotoxin and Glucan react synergistically in kinetic LAL tests to produce enhancement in the interference controls.<sup>2</sup> Therefore, the synergistic activity produces invalid rather than failed results, allowing for a retest with ES Buffer.

### COMPOSITION:

Each vial contains 5.2 mL of carboxymethylated curdlan (CM curdlan) in a pH-buffering solution containing Tris (hydroxymethyl) amino-methane at a pH of 7.4. The buffer is terminally sterilized and is endotoxin-free.

### WARNINGS AND PRECAUTIONS:

1. For in vitro use only. Not to be used in humans or animals.
2. Use this reagent for reconstitution of Endosafe® LAL Reagent only.
3. Do not use this reagent unless it is clear and colorless.
4. ES Buffer contains a large amount of CM curdlan which is  $\beta$ -D-Glucan. CM curdlan acts as a Glucan blocker in the LAL reaction when present in a high concentration.<sup>1</sup> However, CM curdlan in a low concentration is highly reactive to the LAL Reagent. Be careful not to contaminate samples, sterile glassware, LAL Reagent Water, pipets, etc., with ES Buffer.
5. When used to rehydrate End-point Chromogenic LAL Reagent (R160), a stop solution other than acetic acid must be used.

### STORAGE CONDITIONS:

Store all vials at 2 to 8 degrees C. Do not freeze.

### PROCEDURE:

#### Routine LAL-test application.

1. For test materials which have a history of  $\beta$ -D-Glucan or LAL-RM presence, rehydrate the Endosafe® LAL Reagent with ES Buffer to allow LAL testing with greatest endotoxin specificity.
- Note: When using systems where rehydration of the LAL with ES Buffer is not possible, samples can be diluted in a 50% solution of ES Buffer. Take care not to contaminate samples, sterile glassware, LAL Reagent Water, etc., with ES Buffer.
2. For test materials which produce LAL reactivity and where the cause of the response could be due to endotoxin, Glucan, or both, first test the material with Endosafe® LAL Reagent which has been rehydrated with LAL Reagent Water. Next, test the same material with Endosafe® LAL Reagent which has been rehydrated with ES Buffer.
  3. Compare the results of the Glucan-sensitive assay and endotoxin-specific assay for significant differences. The Glucan-sensitive assay yields higher values than the endotoxin-specific assay if samples contain  $\beta$ -Glucan.
  4. Conclude that the implicated lot is free of endotoxin if the results with the endotoxin-specific LAL test is within the endotoxin limit and all criteria for test validity are met.

**ENDOTOXIN-SPECIFIC BUFFER SOLUTION  
(CARBOXYMETHYLATED CURDLAN)**



**Catalog #BG120**

**REFERENCES:**

1. Tsuchiya, M., Takaoka, A., Tokioka, N. and Matsuura, S. Development of an endotoxin-specific Limulus Amebocyte Lysate test blocking  $\beta$ -Glucan-mediated pathway by carboxymethylated curdlan and its application. *Jpn. J. Bacteriol.*, **45**, 903-911 (1990).
2. Cooper, J., M. Weary and F. Jordan. "The impact of non-endotoxin LAL-Reactive Materials on Limulus Amebocyte Lysate analyses." submitted to *PDA J Parent Sci & Tech*, (1996).
3. Guideline on Validation of the LAL Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs. Biological Products and Medical Devices. DHSS, FDA, December 1987.
4. Kakinuma, A., Asano, T., Torii, H. and Sugino, Y. Gelation of Limulus and amebocyte lysate by an antitumor (1-3)- $\beta$ -D-Glucan. *Biochem. Biophys. Res. Commun.*, **101**, 434-439 (1981).
5. Morita, T. "A new (1 $\rightarrow$ 3)- $\beta$ -D-Glucan-mediated coagulation pathway found in Limulus amebocytes." *FEBS Lett.* **129**, 318-321 (1981).
6. Roslansky, P.F. and T. Novitsky. "Sensitivity of Limulus Amebocyte Lysate to LAL-Reactive Glucans." *J Clin Micro* **29** 2477-83 (1991).
7. United States Patent No. 5,179,006. Title of invention: Process for measuring endotoxin. Inventors: Shuji Matsuura, Masakazu Tsuchiya. Assignee: Wako Pure Chemical Industries, Ltd., Osaka, Japan.
8. European Patent No. 330991. Title of invention: Inventors and proprietor are the same as U.S. Patent.

Made by:  
Wako Pure Chemical Industries, Ltd.  
1-2 Doshomachi 3-Chome, Chuo-Ku,  
Osaka 541, Japan

Distributed by: CHARLES RIVER ENDOSAFE  
Div. of Charles River Laboratories, Inc.  
1023 Wappoo Rd., Suite 43-B  
Charleston, SC 29407 USA

PHONE NUMBER: 843-402-4900  
FAX NUMBER: 843-766-7576

PIBG120-02