

GLUCASHIELD®

INTENDED USE

Glucashield is a reconstitution buffer formulated to block interference of (1→3)-β-D-glucans in *Limulus* Amebocyte Lysate (LAL) assays for endotoxin. Neutralization of inhibiting or enhancing substances for the purposes of reducing interference with the LAL assay is permitted under the FDA Guideline on the Validation of the *Limulus* Amebocyte Lysate Test¹ and the USP Bacterial Endotoxins Test².

PURPOSE

Endotoxin test false positives have been associated with the presence of (1→3)-β-D-glucans.^{3,4} The cause of the false positive is the activation of a glucan sensitive factor (Factor G) in LAL.⁵ Glucan contamination may occur from product contact with cellulosic materials or from products derived from fungi. Certain bacterial or algal products may also contribute to glucan-derived interference. Additionally, when interfering glucans are present, test results may be elevated due to enhancement of the response to endotoxin³. Glucashield may be used to block interference caused by glucan concentrations of up to 100 ng/mL (determined with Pachyman, a relatively potent glucan). To determine whether or not the samples are subject to (1→3)-β-D-glucan interference, compare endotoxin levels reported with non-Glucashield reconstituted LAL to those levels obtained with Glucashield reconstituted LAL. If the sample values are significantly lower with Glucashield reconstituted LAL product then it is likely the samples contain (1→3)-β-D-glucans. By blocking these and ensuring that all other required parameters of endotoxin testing are met, it should be possible to successfully measure endotoxin in products that would otherwise be difficult to assay accurately.

REAGENT COMPOSITION

Glucashield consists of an endotoxin-free Factor-G blocking reagent in a 0.2 M Tris-buffered formulation with a pH between 7.6-7.8.

STORAGE CONDITIONS AND WARNINGS

Store at 2-8°C.

Glucashield and Glucashield reconstituted lysate products should not be subjected to freeze/thaw cycles.

Use aseptic technique when using this product.

For *in-vitro* use only. Glucashield is uniquely formulated to be compatible with LAL products manufactured by Associates of Cape Cod. Use of this buffer with LAL products from other manufacturers is not recommended.

INSTRUCTIONS FOR USE

- 1) Use of pliers is recommended for removal of the metal crimp seal from the vial of Glucashield. Carefully remove the stopper from the vial, taking care not to contaminate the contents.
- 2) Reconstitute the LAL Product with Glucashield following the package insert for the specific product using aseptic technique.

For Example:

- a) For Pyrochrome® and Chromo-LAL, the reconstitution volume is 3.2 mL.
- b) For Pyrotell® Gel Clot (5 mL multi-test vials) and Pyrotell®-T Turbidimetric reagents, the reconstitution volume is 5.0 mL.

- 3) Allow the reconstituted LAL product to equilibrate to room temperature prior to use.
- 4) Perform the assay following the instructions in the package insert for the specific product.

PRODUCT NOTES

- 1) Consult the table below for stability of Associates of Cape Cod LAL reagents reconstituted with Glucashield.

LAL Product	Post Glucashield Reconstitution Stability (Hours at 2-8°C)
Pyrochrome	2
Chromo-LAL	6
Pyrotell-T	24
Pyrotell Multitest	24

Note: Post-reconstitution times for reagent reconstituted with Glucashield may differ from those stated in the product inserts. When using Glucashield, the reconstitution times given in the table above apply.

- 2) When using Glucashield in place of Pyrochrome Buffer with Pyrochrome in a kinetic chromogenic test, it may be necessary to extend the incubation time to 75 minutes. This incubation time may need to be determined in your laboratory due to varying incubation conditions.
- 3) Not for use with Pyrotell Single Test Vials (STVs).

REFERENCES

1. 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.
2. Bacterial Endotoxins Test, current USP.
3. Roslansky, P. F. and T. J. Novitsky. 1991. Sensitivity of *Limulus* Amebocyte Lysate (LAL) to LAL-Reactive Glucans. J. Clinical Microbiology 29:2477-2483.
4. Kakinuma, A., T. Asano, H. Torii, and Y. Sugino. 1981. Gelation of *Limulus* amebocyte lysate by an antitumor (1→3)-β-D-glucan. Biochem. Biophys. Res. Commun. 101:434-439.
5. Morita, T., S. Tanaka, T. Nakamura, and S. Iwanaga. 1981. A new (1→3)-β-D-glucan-mediated coagulation pathway found in *Limulus* amebocytes. FEBS Lett. 129:318-321.

Glucashield®

GLUCAN INHIBITING BUFFER

Manufactured by:



124 Bernard E. Saint Jean Drive • E. Falmouth, MA 02536 USA

Telephone: (508) 540-3444
 Toll-Free: (888) 395-2221
 Fax: (508) 540-8680
 Technical Support: (800) 848-3248
 Customer Service: (800) 525-8378