Pharma&Biotech

Lonza

β-G-Blocker

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Content

Important: Read Entire Brochure Before Performing Test

Intended Use

β-G-Blocker is intended as an adjunct for the *In Vitro* end-product endotoxin testing of human and parenteral drugs, biological products, and medical devices using Limulus Amebocyte Lysate (LAL). This product is not indicated as part of a test system for the detection of endotoxin in clinical samples or as an aid in the diagnosis of human disease. β-G-Blocker blocks the reactivity of LAL to β-1,3-Glucans, conferring increased endotoxin specificity to the LAL test. The Pharmacopeial guidelines for LAL testing allows for the neutralization of inhibiting or enhancing substances if the sensitivity of LAL is unaffected[1].

Warning

For *In Vitro* use only. Not for the *In Vitro* determination of Endotoxemia in man. Use this reagent for testing with Lonza LAL Reagent only.

Explanation of Test

The LAL test is an enzyme-mediated assay for the detection of endotoxin[2,3]. This test has largely replaced the rabbit pyrogen test for endotoxin due to the ease of use, cost, sensitivity and specificity of the LAL test. Several factors have been identified that can cause false-positives in the LAL test, i.e. blood products and polynucleotides[4]. Another important source of false-positives involves a class of substances known as β-1,3-Glucans. These substances contain glucose polymers of varying molecular weight linked primarily through β-1,3 glycosidic linkages. If a sufficient amount of β-1,3-Glucans of a particular size class are present, an LAL response may occur that is independent of the endotoxin-mediated response[5,6].

β-G-Blocker is indicated in cases where β-1,3-Glucan contamination is suspected. Examples include preparations derived from yeast and cellulosic materials, including hemodialysis filters. A common dilution/response pattern is seen in the LAL test with samples contaminated with β-1,3-Glucans. This includes a negative response with concentrated samples, a positive response with increasing dilution and eventual negative response at highest dilution. Additionally, with kinetic methods, a synergistic response (i.e. enhancement) is frequently seen with β-Glucan samples spiked with endotoxin.

Principle

β-G-Blocker blocks the glucan-sensitive G pathway in LAL, rendering the test more specific to endotoxin.

Reagents Supplied and Storage Conditions

Reagent (B50-700) Yellow-Labeled Vial

β-G-Blocker, sterile-filtered liquid, 5 × 5 ml/vial, endotoxin-free.

Store at 2–8°C. Product is stable for four weeks once opened if not contaminated and stored at 2–8°C.

Procedure

Follow the LAL test procedure in the package insert for either the Kinetic-QCL™ or PYROGENT™-5000 Assays.

For Use With Kinetic-QCL™ or PYROGENT™-5000 Reagents

If sample dilution is required, first dilute sample with LAL Reagent Water or other suitable diluent. Dilute sample to twice the normal test concentration. For example, if sample is normally diluted 1/10, only dilute to 1/5. After sample dilution has been performed, add one part β-G-Blocker to one part sample. This effectively dilutes the sample 1/2.

Recommended Procedure

To obtain a 1:1 ratio of product to blocker, add no less than 250 µl of test product dilution to 250 µl of blocker in a depyrogenated glass vessel. Vortex this solution and dispense 100 µl to appropriate wells of a 96-well plate. A control (test product with no blocker, same dilution) is required. A positive product control is required with both the control and the product/blocker at each dilution. It is also recommended to test the blocker diluted 1:1 with LAL Reagent Water and a positive product control.

Interpretation of Results

Compare the results of the sample tested with and without blocker. Significantly lower values in samples treated with blocker are indicative of glucan contamination.

Note: The presence of glucan in a sample typically acts synergistically with any endotoxin to produce an LAL response higher than either one alone (an enhancement effect). For example, a sample with glucan (but no endotoxin) may yield a value of 0.4 EU/ml. The same sample spiked to 0.5 EU/ml endotoxin may yield a value of 1.5 EU/ml.

References

1. United States Pharmacopeial Convention. General Chapter <85> Bacterial Endotoxins Test. *United States Pharmacopeia* (USP).

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3. Iwanaga, S. The *Limulus* clotting reaction. *Curr. Opinion in Immunol.* 5:74–82 (1993).

4. Elin, R. and S. Wolff. Nonspecificity of the *Limulus* Amebocyte Lysate test: Positive reactions with polynucleotides and proteins. *J. Infect. Diseases.* 128:349–352 (1973).

5. Morita, T., S. Tanaka, T. Nakamura, and S. Iwanaga. A new (1-3)-β-D-glucan-mediated coagulation pathway found in Limulus amebocytes. *FEBS Lett.* 129:318–321 (1981).

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Certificate of Analysis: www.lonza.com/coa

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