Pharma&Biotech

Lonza

PYROSPERSE™ Dispersing Agent

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Content

Important: Read Entire Brochure Before Performing Test

Intended Use

PYROSPERSE™ Dispersing Agent is intended for use with the PYROGENT™, QCL-1000™ and Kinetic-QCL™ Limulus Amebocyte Lysate (LAL) Assays to assist in the qualitative or quantitative detection of bacterial endotoxin.

Background and Test Principle

Interference with the detection of endotoxin in blood and blood products by the LAL method has been reported by numerous observers[1,2,3,5,7]. Hochstein, *et. al* and others have postulated the existence of an endotoxin binding or masking component which may be present in plasma and blood derivatives[2,7]. Furthermore, studies with other types of pharmaceutical products revealed a similar type of "masking" problem with endotoxin detection. It is postulated that the Lipid A portions of endotoxin molecules, which are essential for LAL activation, can become associated and render the molecule incapable of activating LAL enzyme.

Several publications discuss the use of detergents and surfactants to alter the nature of endotoxin aggregates, but each of those reported has been shown to be incompatible with the LAL test method[4,6,8].

PYROSPERSE™ Dispersing Agent is one of a group of metallo-modified polyanionic dispersants which has proven useful as a sample modifying agent for certain types of products showing inhibition in the LAL assay[9].

In those products for which endotoxin binding is the suspected source of inhibition, the use of PYROSPERSE™ Dispersing Agent should be considered. To date, PYROSPERSE™ Dispersing Agent has been found useful in LAL endotoxin detection when used with the following products: Human Serum Albumin, 5% and 25%; Plasma Protein Fraction; Electrolyte solutions; Antihemophilic Factor; and Lipid emulsions. Additional product applications may exist.

Reagents Supplied and Storage Conditions

Reagent (F188) Yellow-Labeled Vial

PYROSPERSE™ Dispersing Agent is an endotoxin-free solution of a metallo-modified polyelectrolyte that has been prepared in water for injection. It contains no preservatives.

Reagent	Quantity	Concentration
PYROSPERSE™ Dispersing Agent	5 × 5 ml/vial	22% (W/V Solution)

Precautions

– For use in conjunction with the PYROGENT™, QCL-1000™ and Kinetic-QCL™ LAL Assays.
– Successful performance of this test requires strict adherence to all items in the procedure recommended herein and the appropriate package insert.
– All materials coming in contact with the samples or test materials must be endotoxin-free.

Storage

Unopened vials of PYROSPERSE™ Dispersing Agent may be stored at 2–30°C for no longer than the labeled expiration date.

Once a vial has been opened, it should be stored at 2–8°C for no longer than four weeks. Refrigerated samples should be brought to room temperature (15–30°C) before use.

Indications of Deterioration

PYROSPERSE™ Dispersing Agent which does not appear as a clear, amber solution should be discarded.

Sample Collection and Preparation

Samples must be collected and prepared using depyrogenated equipment and endotoxin-free reagents.

Samples should be checked to insure that the pH is within the range of 6.0–8.0. If pH adjustment is necessary, endotoxin-free sodium hydroxide or hydrochloric acid solution of an appropriate concentration should be used prior to the addition of PYROSPERSE™ Dispersing Agent. Always measure the pH of an aliquot of the bulk sample to avoid contamination by the pH electrode. Do not adjust the pH of unbuffered saline, water, or solutions.

The use of PYROSPERSE™ Dispersing Agent should be considered when a substance is suspected of causing inhibition of the LAL reaction due to an endotoxin binding or masking component. The degree of inhibition should be determined using procedures described in the respective LAL product package inserts. The following is a brief summary of these procedures.

Using PYROGENT™

Assay a dilution of product plus endotoxin in conjunction with dilution series of water plus endotoxin. If the minimum detectable endotoxin concentration in the product is within 2-fold of the minimum detectable level of endotoxin in water, the product may be considered to be non-inhibitory.

Using QCL-1000™ or Kinetic-QCL™

Assay an aliquot of product (or a dilution of product) spiked with a known amount of endotoxin along with the unspiked product to determine their respective endotoxin concentrations. If the difference between these two calculated endotoxin values is equal to the known concentration of the spike, within acceptable limits, the product may be considered non-inhibitory.

Test Procedure

PYROSPERSE™ Dispersing Agent is used in the sample preparation prior to the LAL test. Sample preparation may be performed in accordance with the general procedures described below. The LAL test should be performed in strict accordance with the test procedure described in the package insert accompanying the LAL test kit.

Specific test procedures must be determined by the user for each type of product.

Sample Preparation

PYROSPERSE™ Dispersing Agent is added to test samples prior to LAL testing. The following procedures are recommended for testing Human Serum Albumin, Plasma Protein Fraction, Electrolyte solutions, Anti-hemophilic Factor, and Lipid emulsions. Use of PYROSPERSE™ Dispersing Agent for sample preparation in other products may require different concentrations of dispersing agent. These concentrations should be determined experimentally by the user. However, studies to date show that concentrations of PYROSPERSE™ Dispersing Agent greater than or equal to 1/2.5 (V/V) are not indicated for use in LAL testing.

Using PYROGENT™

1. Carefully, to avoid bacterial and endotoxin contamination, add 0.1 ml of PYROSPERSE™ Dispersing Agent to 5.0 ml of sample or sample dilution. If other than 5.0 ml of sample is used, the amount of PYROSPERSE™ Dispersing Agent added should be adjusted so that the resulting concentration of PYROSPERSE™ Dispersing Agent is 1/50 (V/V) in the sample mixture.

2. Vortex mixture for a minimum of 15 seconds.

3. Conduct LAL tests in accordance with package insert accompanying the LAL test kit.

Using QCL-1000™ or Kinetic-QCL™

1. Add 0.025 ml of PYROSPERSE™ Dispersing Agent to 5.0 ml of sample or sample dilution. If other than 5.0 ml of sample is used, the amount of PYROSPERSE™ Dispersing Agent added should be adjusted so that the resulting concentration of PYROSPERSE™ Dispersing Agent is 1/200 (V/V) in the sample mixture.

2. Vortex mixture for minimum of 15 seconds.

3. Conduct LAL tests in accordance with package insert accompanying the LAL test kit.

Note: For QCL-1000™, sodium dodecyl sulfate (10g/100ml) should be used as the stop reagent.

Utilization of PYROSPERSE™ Dispersing Agent in sample preparation for both the qualitative and quantitative LAL assay should show an improvement in endotoxin recovered. If no improvement is demonstrated, PYROSPERSE™ Dispersing Agent is not recommended.

Limitations and Indications

Potential interference by products to be tested must be determined as previously stated. The optimal concentration of PYROSPERSE™ Dispersing Agent for any given product may need to be adjusted above or below that suggested. Studies have shown that concentrations of PYROSPERSE™ Dispersing Agent greater than 1/2.5 (V/V) are not indicated for use in LAL testing.

References

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Certificate of Analysis: www.lonza.com/coa

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