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LAL Reagents

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LAL Methodology & Applications

Introduction to the LAL Test
Limulus Amebocyte Lysate (LAL) tests detect and quantify bacterial endotoxins extracted from the outer wall of gram negative bacteria. The critical component of the LAL reagents used in endotoxin tests is derived from blood cells (amebocytes) of the horseshoe crab, Limulus polyphemus. It contains the proteins of the blood clotting mechanism, which is triggered by endotoxins. LAL reagents are primarily used to test for endotoxins in injectable pharmaceuticals, biological products, and medical devices. They are also used in renal dialysis centers and a wide range of other applications. LAL tests are described in the Bacterial Endotoxins Test chapter in the United States Pharmacopeia (Chapter <85>) and in the equivalent chapters in the European Pharmacopoeia (Chapter 2.6.14) and the Japanese Pharmacopoeia (General Tests, No. 4.01).

LAL Test Methods
There are three principal LAL test methods: the gel-clot, turbidimetric and chromogenic methods. The latter two may be grouped together as photometric methods as they require an optical reader.

Chromogenic Method
The LAL reagent is formulated with a synthetic substrate which produces a chromophore when acted upon by endotoxin activated enzyme. The test is read at 405 nm, usually in a microplate reader. The maximum sensitivity is 0.005 EU/mL.

Turbidimetric Method
This is most sensitive LAL test available and the maximum sensitivity of 0.001 EU/mL can be achieved when run in our Pyros Kinetix® tube reader (See page 5.2). The optical density (turbidity) increase that accompanies the clotting reaction is read in the Pyros Kinetix® tube reader or in a incubating microplate reader.

Gel-clot Method
This is simplest LAL test. The formation of a gel-clot indicates the presence of endotoxin in a sample. The method is performed in small test tubes and is read manually by inverting the test tubes. The maximum sensitivity is 0.03 EU/mL.

Selecting an LAL method?
When deciding which LAL test method is to be used, the following questions can be asked:
- What is the available budget?
- What are the regulatory requirements, if any?
- What type of product or sample is going to be tested?
- What test sensitivity is required?
  (What is the endotoxin limit specification for the sample?)
- Is electronic storage of data desired?

If the budget is limited, the gel-clot method is the least expensive method as no optical reader is required. Also, the gel-clot method may be the method of choice for opaque samples, suspensions or colored samples, though dilution of the sample may enable use of the photometric methods. For users with a non-incubating optical reader, an endpoint chromogenic test may be the best choice. As with other photometric methods, this will provide printouts of results and electronic storage of data. The kinetic methods (chromogenic and turbidimetric) provide the widest detection range and sophisticated software. The photometric methods offer the greatest sensitivity, allowing detection of low endotoxin concentrations and greater dilutions of sample, which is important for overcoming interference.

If testing with a licensed reagent is not a requirement, we offer additional testing options, including endotoxin and glucan specific reagents.

The merits of the various methods are summarized below:

Chromogenic Method
- Requires a microplate reader (an incubating reader is required for the kinetic method)
- Maximum sensitivity to 0.005 EU/mL
- A quantitative assay providing electronic stored data and print-outs of results
- Incubation time varies depending on the standard curve range
- Higher sensitivity allows for greater dilution to overcome interference
- The option of a diazo-coupled endpoint method (read at 540–550 nm) is available, which is useful for samples that absorb light at 405 nm
Product Characterization

Samples should be characterized for endotoxin contamination and/or potential interference. Characterization is not a regulatory requirement, but is important to develop a test method that can be validated to demonstrate the absence of interference. It is typically performed by testing a series of dilutions of sample without and with a known amount of added endotoxin (Positive Product Control or PPC). The purpose of the PPC is to indicate that added endotoxin is appropriately detected and that the sample does not interfere with the test. From the results of characterization testing, a product dilution (and possibly product treatment) is selected for validation of the test (see below). The endotoxin limit for the product must be detectable at the dilution selected.

Test for Interfering Factors (Validation)

The test for interfering factors is performed to validate the test for the particular sample type. It is accomplished by demonstrating, with three lots of product, that endotoxin added to the sample in PPCs can be readily detected within required limits.

Routine Testing

Routine testing is conducted at the validated dilution and includes a parallel PPC to control for interference. Tests should also include negative controls and appropriate standards. A minimum of three units per lot of drug product should be tested, with the samples taken from the beginning, the middle and the end of the production run. For medical devices, aqueous extracts of up to ten units are tested, usually after pooling.

Conclusion

The preceding overview is just the beginning. Further assistance with selecting a test method or reagent sensitivity is always available from our technical support personnel, and representatives in the field. Our staff and distributors can help with characterization, validation or routine testing. The LAL Update, our newsletter, includes useful technical articles and is available on the website. Our Contract Test Service (see page 6.1) regularly assists with characterization and method development and can provide results by all test methods. Which ever method is selected, you can always be assured of the full support of Associates of Cape Cod, Inc.

For details on the regulatory requirements for compliant LAL testing in the United States (US), users should consult the current revision of the United States Pharmacopia (USP), chapter <85>, “Bacterial Endotoxins Test.” Additional information is available in the US Food and Drug Administration’s 1987 guidance document “Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices.” (available online at http://www.fda.gov/cder/guidance/old005fn.pdf).
Kinetic Turbidimetric Method
An Economical, Flexible & Sensitive Solution

Introduction
Pyrotell®-T
Turbidimetric Formulation

General Product Description
Pyrotell®-T lysate is used to quantify endotoxin in kinetic turbidimetric tests. Pyrotell-T lysate can be used with the Pyros Kinetix® tube reader and also incubating microplate readers. When used with the Pyros Kinetix tube reader, Pyrotell-T gives a highly economic, flexible and sensitive LAL assay.

It can be used for a wide variety of endotoxin tests, ranging from standard water testing to samples requiring high sensitivity, such as intrathecal products and those requiring high dilutions to overcome interference.

Sensitivity
Pyrotell®-T lysate is the most sensitive LAL test available, which is 5 times more sensitive than any other LAL assay. When used in a Pyros Kinetix® tube reader the maximum sensitivity is 0.001 EU/mL. The unique formulation of Pyrotell-T lysate allows a wide range of standard curves to be used, giving the user flexibility, speed, and ease in performing assays. When used in a microplate reader, the maximum sensitivity is 0.005 EU/mL.

Sample to Lysate Ratio
The ratio of sample to LAL is determined by personal preference and sample chemistry (interference patterns). A sample to LAL ratio for Pyrotell-T of 1:1 or 4:1 can be used according to user choice (and validation requirements).

Performing the Test
The sample and lysate are added to test tubes or a microplate and incubated in an optical reader at 37°C. The time of incubation is dependent on the lowest standard concentration in the standard curve. Software is used to construct the standard curve and calculate the endotoxin concentrations.

Reconstitution
Pyrotell-T lysate may be reconstituted with 5mL of LAL Reagent Water (LRW) (or equivalent), Pyrosol® buffer, or Glucashield® buffer, depending on the demands of the sample being tested. Pyrosol buffer provides extra pH buffering capacity. Glucashield buffer, a beta glucan inhibiting buffer is used to render the assay endotoxin specific.

Stability
Once reconstituted, Pyrotell-T lysate is stable for 24 hours, if stored at 2–8°C. Pyrotell-T lysate may be frozen once and will retain activity for as long as 3 months if stored at or below -20°C.

Packaging
Pyrotell-T lysate is available in individual multi-test vials. Each vial contains reagent for approximately 96 tests (when used with the Pyros Kinetix tube reader and 4:1 ratio only) or 48 tests (when used in a microplate reader).

Accessory Products
1. LAL Reagent Water, available in multiple package sizes, see page 4.2
2. Control Standard Endotoxin, E. coli O113:H10, 0.5 µg per vial (E0005)
3. Glucashield® Buffer (GB051), (1→3)-β-D-Glucan Inhibiting Buffer
4. Pyrosol® LAL Reconstitution Buffer (BC051)
5. Pyrotubes® (TK100), 8 x 75 mm borosilicate glass test tubes
6. Pyrotubes® (TB013), 13 x 100 mm borosilicate dilution tubes
7. Pyroplate® (CA961), 96-well microplate
8. 250 µL Pipette tips (PPT25), endotoxin and glucan-free
9. 1000 µL Pipette tips (PPT10), endotoxin and glucan-free
10. Pyros Kinetix® incubating kinetic tube reader (PKX02)
11. Incubating microplate reader, see pages 5.4 and 5.6

For more information please visit our website at www.acciusa.com or contact Technical Services at (800) 848–3248.
Kinetic Chromogenic Method
Stable & Robust Quantitative Testing

Introduction

VersaMax Incubating Microplate Reader
**Chromo-LAL**

**Kinetic Chromogenic Formulation**

**General Product Description**
Chromo-LAL lysate is lyophilized with substrate reagent and buffers. It is optimized for the kinetic chromogenic LAL test method in microplate readers. Chromo-LAL is a buffered, stable and robust lysate, suitable for quantitative testing of a wide range of samples.

**Sensitivity**
The sensitivity for chromogenic assays is determined by the lowest standard concentration on the standard curve used for the assay. The maximum sensitivity of Chromo-LAL is 0.005 EU/mL.

**Sample to Lysate Ratio**
In the Chromo-LAL test, reconstituted Chromo-LAL reagent is used at a ratio of 1:1 and a volume of 100 µL.

**Performing the Test**
The Chromo-LAL/sample mixture is incubated at 37 ± 1°C in a microplate reader equipped with a 405 nm filter. Software is used to construct the standard curve and calculate the endotoxin concentrations.

**Reconstitution**
Chromo-LAL lysate is reconstituted with LAL Reagent Water (LRW). It can also be reconstituted with Glucashield® buffer, a beta glucan inhibiting buffer, to render the assay endotoxin specific.

**Stability**
The reconstituted lysate is stable for 24 hours at 2–8°C or for two weeks at -20°C or colder if frozen immediately after reconstitution. Reconstituted Chromo-LAL lysate may be frozen and thawed once.

**Packaging**
Chromo-LAL lysate is sold by the vial for greater ordering and testing flexibility. This allows you to order the amount of lysate that is right for you.

**Accessory Products**
1. LAL Reagent Water, available in multiple package sizes, see page 4.2
2. Control Standard Endotoxin, *E. coli* O113:H10, 0.5 µg per vial (E0005)
3. Glucashield® Buffer (GB051), (1→3)-β-D-Glucan Inhibiting Buffer
4. Pyrotubes® (TB013), 13 x 100 mm borosilicate glass dilution tubes
5. Pyroplate® (CA961), 96-well microplate
6. 250 µL Pipette tips (PPT25), endotoxin and glucan-free
7. 1000 µL Pipette tips (PPT10), endotoxin and glucan-free
8. Incubating microplate reader

For more information please visit our website at www.acciusa.com or contact Technical Services at (800) 848-3248.
Pyrochrome®
Chromogenic Endotoxin Testing

General Product Description
Pyrochrome is a versatile quantitative chromogenic reagent that may be used to perform either kinetic or endpoint assays in microplate readers. The standard Pyrochrome test is read at 405 nm.

Pyrochrome is also offered in a diazo kit for endpoint tests. The diazo reagents shift the optical absorbance wavelength making it especially useful for testing samples with color interference. The test is read at 540–550 nm.

Sensitivity
The sensitivity for chromogenic assays is determined by the lowest standard concentration on the standard curve used for the assay. The maximum sensitivity of Pyrochrome is 0.005 EU/mL.

Sample to Lysate Ratio
In the Pyrochrome test, reconstituted Pyrochrome LAL reagent is used at a ratio of 1:1 and a volume of 50 µL or 100 µL.

Performing the Test
The Pyrochrome/sample mixture is incubated at 37±1°C and read in a microplate reader equipped with a 405 nm filter or 540–550 nm filter for diazo method. Software is used to construct the standard curve and calculate the endotoxin concentrations.

Stability
Pyrochrome must be used within 8 hours of reconstitution. Vials may be stored at 2–8°C (or on ice). Reconstituted Pyrochrome can not be frozen.

Reconstitution
Pyrochrome lysate is reconstituted with an optimized reaction buffer.

Pyrochrome can also be reconstituted with Glucashield® buffer, a beta glucan inhibiting buffer, to render the assay endotoxin specific.

Packaging
Pyrochrome® product line is offered with a matched buffer and is recommended for use with the 10 ng/vial Control Standard Endotoxin (EC010).

Pyrochrome® is also offered in a diazo kit for end-point tests. The diazo reagents change the absorption maximum from 405 nm to 540–550 nm making it especially useful for testing samples with color interference.

Accessory Products
1. LAL Reagent Water, available in multiple package sizes, see page 4.2
2. Control Standard Endotoxin 10 ng/vial (EC010), E. coli O113:H10
3. Glucashield® Buffer (GB051), (1→3)-β-D-Glucan Inhibiting Buffer
4. Pyrotubes® (TB013), 13 x 100 mm borosilicate glass dilution tubes
5. Pyroplate® (CA961), 96-well microplate
6. 250 µL Pipette tips (PPT25), endotoxin and glucan-free
7. 1000 µL Pipette tips (PPT10), endotoxin and glucan-free
8. Incubating microplate reader

For more information please visit our website at www.acciusa.com or contact Technical Services at (800) 848–3248.

Pyrochrome®
#C1500 ........................................... 60 Test
#CD060 ........................................... 60 Test
(with Diazo-reagents)
Kinetic Chromogenic Method
Stable & Robust Quantitative Testing
Gel-Clot Method

The Industry Standard

Introduction
Pyrotell®

Gel-clot Formulation

General Product Description
Pyrotell® lysate was the first LAL reagent licensed by the US FDA. It is easy to use and is available in both economical multi-test vials and convenient Single Test Vials (STVs). Pyrotell lysate is a robust reagent, producing firm, easily read clots and is resistant to interfering substances. The gel-clot test does not require sophisticated capital equipment and software and is the simplest LAL test to implement.

Sensitivity
Pyrotell lysate is available in a variety of sensitivities: 0.03 EU/mL; 0.06 EU/mL; 0.125 EU/mL; 0.25 EU/mL. 0.5 EU/mL available in Single Test Vials only.

Sample to Lysate Ratio
For standard use, a 1:1 mixture of Pyrotell lysate to sample is used.

Performing the Test
For 2 mL and 5 mL multi-test vials, 100 µL of lysate is mixed with 100 µL of sample in a reaction tube. For single test vials 200 µL of sample is added to the vial, which serves as a reaction tube. Test tubes are incubated at 37°C for 60 minutes ± 2 minutes. A positive test is indicated if the clot remains solid after the inversion of the test tube.

Reconstitution
Pyrotell multi-test vials may be reconstituted with LAL Reagent Water (LRW), Pyrosol® buffer or Glucashield® buffer. Glucashield buffer, a beta glucan inhibiting buffer is used to render the assay endotoxin specific.

Stability
The reconstituted lysate is stable for 24 hours at 2–8°C or for up to 3 months at or below -20°C. May be frozen once and will retain activity for as long as 3 months if stored at or below -20°C.

Packaging
Pyrotell lysate is available in single test vial (STV), 2 mL, or 5 mL fill sizes. The 2 mL and 5 mL vials are sold individually. STVs are sold in packs of ten vials. Control Standard Endotoxin (CSE) is provided separately with a Certificate of Analysis, specific to the Pyrotell lysate lot with which it will be used.

Accessory Products
1. LAL Reagent Water, available in multiple package sizes, see page 4.2
2. Control Standard Endotoxin, *E. coli* O113:H10, 0.5 µg per vial (E0005)
3. Glucashield® Buffer (GB051), (1→3)-β-D-Glucan Inhibiting Buffer
4. Pyroso® Buffer with pH indicator (BR051)
5. Pyrosol LAL Reconstitution Buffer (BC051)
6. Pyrotubes® (TB050), 10 x 75 mm soda lime glass test tubes
7. Pyrotubes® (TB240), 12 x 75 mm borosilicate dilution tubes
8. Pyrotubes® (TB013), 13 x 100 mm borosilicate dilution tubes
9. 250 µL Pipette tips (PPT25), endotoxin and glucan-free
10. 1000 µL Pipette tips (PPT10), endotoxin and glucan-free

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Multi-Test, 5 mL/vial
#G5003 ----------------------- 0.03 EU/mL
#G5006 ----------------------- 0.06 EU/mL
#G5125 ---------------------- 0.125 EU/mL
#G5250 ----------------------- 0.25 EU/mL
#GP5003 ---------------------- 0.03 EU/mL (sold with Pyrosol)

Multi-Test, 2 mL/vial
#G2003 ----------------------- 0.03 EU/mL
#G2006 ----------------------- 0.06 EU/mL
#G2125 ---------------------- 0.125 EU/mL
#G2250 ----------------------- 0.25 EU/mL

Single Test Vial (STV) 0.2 mL/vial, 10 vials/pack
#G5003 ----------------------- 0.03 EU/mL
#G5006 ----------------------- 0.06 EU/mL
#G5125 ---------------------- 0.125 EU/mL
#G5250 ----------------------- 0.25 EU/mL
#G5500 ----------------------- 0.5 EU/mL
Control Standard Endotoxin (CSE)

General Product Description
Control Standard Endotoxin (CSE) is a widely used standard for endotoxin testing. It is a purified extract from *E. coli* O113:H10, the same strain used for the United States Pharmacopeia and the European Pharmacopeia reference standard endotoxin (RSE).

CSE is used as an economic alternative to the RSE. CSEs are standardized against the RSE as indicated on the Certificate of Analysis, so that results can be reported in Endotoxin Units (EU) and International Units (IU). CSE can be used for all LAL testing. A 10 µg/vial CSE is made specifically for use with our Pyrochrome chromogenic reagent.

Depyrogenation Controls
In addition to their use as standards for controlling LAL tests, the 0.5 µg and 125 µg CSEs can be used for validation of depyrogenation processes. They may be used directly, without reconstitution, as depyrogenation indicators (recommended for 0.5 µg) or can be reconstituted and endotoxin added to challenge articles (recommended for 125 µg).

Performing the Test
CSE is used to make standard curves and controls when performing the LAL assay. The concentrations used are dependent on the type of assay (e.g., chromogenic, gel-clot, or turbidimetric) and for photometric methods, the detection range required.

Reconstitution
CSE is reconstituted with LAL Reagent Water (LRW) (or equivalent). Please refer to the Certificate of Analysis when using CSE. A Certificate of Analysis exists for each CSE-lysate lot combination.

Stability
CSE is reconstituted with LRW or equivalent, and is stored at 2–8°C before and after reconstitution. Once reconstituted, the maximum storage time for the different CSE preparations is listed below.

- 10 ng/vial  - 7 days
- 0.5 µg/vial  - 4 weeks
- 125 µg/vial  - 3 months

CSE should not be frozen.

Packaging
The most commonly used size is 0.5 µg/vial. The 0.5 µg/vial is also used for testing depyrogenation ovens. The 125 µg/vial is typically used for special purposes, e.g., making endotoxin-challenge articles for validation of depyrogenation processes. The 10 ng/vial CSE is available in packs of five vials.

Product Benefits
- CSE is a premium, stable, preparation of endotoxin that can be used in all LAL testing
- CSE is especially useful for depyrogenation studies
- Certificates of Analysis for each CSE-lysate lot pairing gives a potency that is specific to the unique lot combination
- CSE can be reconstituted to achieve specific endotoxin concentrations

For more information please visit our website at www.acciusa.com or contact Technical Services at (800) 848–3248.

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#E0005 - Escherichia coli O113:H10, 0.5 µg/vial
#E0125 - Escherichia coli O113:H10, 125 µg/vial
#EC010 - Escherichia coli O113:H10, 10 ng/vial, 5 vials per pack, for use with Pyrochrome®
#PC010 - Positive Control for Single Test Vial, 0.2 mL LAL and CSE, 10 vials per pack, CSE concentration is 0.1 ng/mL, (for research use only)