

Serum/Plasma Testing with PYROGEN[®] 5000 and Kinetic-QCL[®] Assays

Technical Tips

By Scientific Support, U.S.

The following procedure can be used to prepare serum or plasma for LAL-based endotoxin testing. Plasma or serum can be tested for endotoxin after some preliminary preparation. If testing plasma, one should use “platelet rich plasma” which can be obtained from whole blood by centrifuging at low speeds to remove the white and red blood cells. Blood serum products, whole blood processed to remove fibrogens, coagulants, whole blood cells, etc., can also be tested in the same manner as blood plasma.

Procedure

1. Dilute plasma or serum 1:10 with LAL Reagent Water (LRW).
 - a. Example: Add 0.1 mL plasma or serum to 0.9 mL LRW.
 - b. Note: 1:10 dilution is a good starting point. Adjusting this dilution may be required, but this number should not be less than 1:3, as the sample may coagulate at lower dilutions.



2. Heat-inactivate the 1:10 dilution of plasma or serum by placing the sample in a water bath or heat block at 70°C for a minimum of 15 minutes.

⚠ Some samples may require more/less incubation time and/or higher/lower incubation temperature for optimal Positive Product Control (PPC) recovery and endotoxin detection. 15 minutes at 70°C is a good starting point.

3. Next, make a series of 1:2 dilutions with LRW of the 1:10 diluted and heat-treated sample.

Dilution	Vol. of Preceding Dilution	Vol. of LAL Reagent Water
1:20	0.5 mL	0.5 mL
1:40	0.5 mL	0.5 mL
1:80	0.5 mL	0.5 mL
1:160	0.5 mL	0.5 mL
1:320	0.5 mL	0.5 mL

4. PYROGENT® 5000 is the preferred assay for testing most blood product samples, as we have found there is less interference. The Kinetic-QCL® Assay can also be used, however, the dilution will need to be higher to achieve similar PPC recovery.

5. If the sample has been collected with an anticoagulant such as heparin or EDTA, the use of MgCl₂ solution (Lonza Catalog Number: S50-641) may be required to overcome the chelation effect of these anticoagulants. In this case, the first 1:10 dilution prior to heating (Step #1) should still be done with LRW, while subsequent dilutions (Step #3) should be done with 10 mM MgCl₂ solution.

6. Run the LAL endotoxin test according to the directions supplied in the appropriate Product Insert. Run each dilution of plasma or serum in duplicate and include a PPC for each dilution. If using the Kinetic-QCL® Assay, a PPC is defined as 0.5 EU/mL or 5.0 EU/mL, depending on the product dilutions' pass/fail cutoff criteria. If using the PYROGENT® 5000 Assay, a PPC is defined as 0.1 EU/mL or 1.0 EU/mL.

⚠ In an environment such as R&D or non-FDA regulated product testing, the PYROGENT® 5000 Assay may be run with a lowest standard of 0.001 EU/mL. Running the assay to this sensitivity is possible if all conditions are tightly controlled. This will allow endotoxin detection to the level some researches are attempting to achieve.

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