

PENTAPHARM

Pefachrome[®] LAL

Application: Highly sensitive chromogenic peptide substrate for the determination of bacterial endotoxins.

Formula: CH₃SO₂-D-CHA-Gly-Arg-pNA-AcOH **MW:** 642.7

Storage: May be used by the expiry date given on the label when stored unopened, protected from moisture, in the dark, 2-8°C. Avoid contamination of the reagents by micro-organisms. Shipment of product does not require cooling during the time of transportation.

Principle:
a) Proenzyme + Endotoxin ==> Active Enzyme (AE)
b) CH₃SO₂-D-CHA-Gly-Arg-pNA + AE ==> CH₃SO₂-D-CHA-Gly-Arg-OH + pNA + AE

Test procedure:**Material required and preparation:**

Endotoxin standard (Byk-Sangtec), 100 EU to be dissolved in 5 ml water
LAL-Reagent, sensitivity: 0.03 EU/ml
Pyrogen-free water
Pefachrome[®]LAL, dissolve 25 mg in 23,71 ml water and dilute 1:2 with buffer before use in the LAL-test.
Buffer: 0.05 M Tris/HCl pH 9.0 with 0.2 M NaCl
Acetic acid 20%

Method for micro-titer plates

Standard dilutions for the test:
1.0 - 0.5 - 0.25 - 0.125 - 0.0625 - 0.031 - 0.015 EU/ml as well as blank

50 µl lysate 50 µl standard or sample ⇒ incubate for 20 min at 37 °C 100 µl substrate-buffer solution ⇒ incubate for 3 min at 37 °C 200 µl acetic acid ⇒ read OD at 405 nm
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Plasma samples (heparin plasma, 15 IU heparin/ml whole blood) are diluted 1 : 10 with water and heated for 10 min at 75 °C.

Moreover, the standard curve in plasma has to be established.

The sensitivity of the test is approximately 4 times higher in plasma than in water. The required quantities correspond to those mentioned above; incubation of sample and lysate should last to 35 min.

Plasma is considered as pyrogen-free if the blank value shows an ΔOD less than 0.15.

Gel method

After reconstitution, LAL should be aliquoted aseptically into pyrogen-free 10 x 75 mm test tubes, 0.1 ml per tube. Add 0.1 ml of the test solution (pre-warmed to 37 °C pH 6- 7.5) to the test tube. If a negative control is needed, add 0.1 ml of sterile, pyrogen-free water to a test tube. Swirl gently to mix, and place the tubes vertically into a 37 °C water bath for one hour. Do not move the tubes to prevent disturbance of gel formation.

Package size: Vial containing 25 mg
Bulk [g]

Code: 086-06

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