

0.25 M TRIS BASE SOLUTION (TRIS {HYDROXYMETHYL} AMINOMETHANE)

INTENDED USE:

The 0.25 M Tris Base Solution is used as an adjunct for endotoxin testing by Limulus Amebocyte Lysate (LAL) methods. The principle use of this solution is to buffer acidic solutions prior to LAL testing.¹ The USP Bacterial Endotoxins Test <85> for LAL test applications allows the use of adjuncts to overcome inhibitory LAL-test conditions.²

EXPLANATION OF TEST:

The LAL test is the most sensitive and specific means available to detect and measure bacterial endotoxin. The LAL reaction is an enzyme-mediated process which requires a neutral pH environment and a proper balance of monovalent and divalent cations.^{1,2} A pH-related inhibition is likely when the pH is lower than the optimum range and when there is a failure to recover the positive product control in the LAL test. With the addition of the 0.25 M Tris Base Solution to an acidic test solution, the pH of the material may be brought to neutrality by a gradual process.

The most common type of LAL-test interference is sub-optimum pH conditions.¹ The ideal method to resolve pH-related interference is to use permissible dilution. Since the Endosafe® LAL Reagent is well buffered, neutralization of the test sample may not be needed after test-sample dilution, even though the material is known to be acidic, beforehand. However, when levels of interfering components in the test sample are high and the maximum valid dilution (MVD) is low, the use of additives is the only option to testing within the permissible dilution. Refer to the procedure section for proper use of the 0.25 M Tris Base Solution.

COMPOSITION:

Each vial contains 5 mL of a pH-buffering solution containing a 0.25 Molar concentration of Tris (hydroxymethyl) aminomethane with a pH of approximately 9. The buffer is terminally sterilized and is endotoxin-free.

WARNINGS AND PRECAUTIONS:

1. For in vitro use only. Not to be used in humans or animals.
2. Only use this reagent for pH neutralization of solutions being prepared for a LAL test using Endosafe® LAL Reagent. Do not use this buffer to rehydrate Endosafe® LAL Reagent.
3. Do not use this reagent unless it is clear and colorless.
4. Only use pH-measuring electrodes that are compatible with Tris solutions.

STORAGE CONDITIONS: Store solution at room temperature. Do not freeze.

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PROCEDURES:

Determination of the pH of the sample-LAL mixture.

1. Dilute the material to be tested to the desired concentration, within the MVD.
2. Mix equal parts of diluted test solution and Endosafe® LAL in a depyrogenated tube.
3. Check the pH of the mixture with a Tris-compatible system. The test material may be incompatible with LAL methods if the pH is not in the optimum range of 6.5 to 8.0.
4. Add suitable aliquots of the 0.25 M Tris Base Solution to the test material until the LAL-sample mixture is both in the optimum pH range and compatible with the selected LAL method.

Routine LAL-test application.

1. If pH adjustment is required for routine LAL testing, conduct a validation study to confirm the amount of 0.25 M Tris Base Solution to be added per unit amount of test material.
2. Ideally, add the 0.25 M Tris Base during the first dilution step to maximize the efficiency of the neutralization procedure. The combination of dilution and first-step buffering will minimize both the amount of required buffer and dilution of the test material.
3. To test under conditions that are consistent with the USP Bacterial Endotoxins Test <85>², the pH must be measured and recorded for a routine LAL test which requires a pH-neutralized procedure.
4. For raw materials such as strong organic acids or very acidic solutions, it may be necessary to dissolve or pre-treat the test material with 0.1 N NaOH before neutralization with 0.25 M Tris Buffer Solution.

REFERENCES:

1. Cooper, J.F. "Resolving LAL Test Interferences." J. Parent. Sci. & Tech., 44:1, p.13 (1990).
2. Bacterial endotoxins test <85>. In The U.S. Pharmacopeia, 37th rev., United Book Press Inc., Baltimore, MD.

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