

0.5 M MgSO₄ IN 1.0 M TRIS BUFFER SOLUTION (TRIS {HYDROXYMETHYL} AMINOMETHANE)**INTENDED USE:**

The 0.5 M MgSO₄ in 1.0 M Tris Buffer Solution is used as an adjunct for endotoxin testing by Limulus Amebocyte Lysate (LAL) methods. The principle use of this solution is to provide divalent-cation replacement and pH buffering to test-materials 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 (PPC) in the LAL test. If divalent cations in the LAL Reagent are depleted because of the presence of high concentrations of chelating agents, such as > 0.1 M citrate, there will be a failure to recover the PPC, as well. With the addition of magnesium sulfate and Tris buffer solution, the optimum reaction conditions for the LAL reaction may be restored.

The most common types of LAL-test interference are sub-optimum pH and divalent-cation depletion conditions.¹ The ideal method to resolve chemical inhibition is to use permissible dilution. When levels of interfering components in the test sample are high and the maximum valid dilution (MVD) is low, the use of additives are the only option to testing within the permissible dilution. See use instructions below.

COMPOSITION:

Each vial contains 4 mL of 0.5 M MgSO₄ in a pH-buffering solution containing a 1.0 Molar concentration of Tris (hydroxymethyl)-aminomethane with a pH of 6.8 to 7.6. 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 and Mg replacement of solutions being prepared for 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 electrodes that are compatible with Tris solutions.

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

PROCEDURES:

Determination of the pH of the sample-LAL mixture.

1. Dilute the material to be tested to the desired concentration.
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 this Mg/Tris solution to the test material until the LAL-sample mixture is in the optimum pH range and is compatible with the selected LAL method.

Routine LAL-test application.

1. If Mg replacement and pH adjustment are required for routine LAL testing, conduct a validation study to confirm the volume of Mg/Tris solution to be added per unit amount of test material.
2. Ideally, add the Mg/Tris solution during the first dilution step to maximize the effect of the magnesium-replacement buffer.
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.

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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