

Alternative growth medium

Modified TB2 medium

Sea salt (Sigma)	40.0	g
KH ₂ PO ₄	0.1	g
NH ₄ Cl	0.25	g
KNO ₃	1.0	g
MES	5.0	g
Na ₂ S-9H ₂ O	0.5	g
Resazurin	1.0	mg
Distilled water	1.0	L

Mix ingredients, except KNO₃, MES and Na₂S-9H₂O, bring to boil, and cool under a H₂-CO₂ (4:1, v/v) gas mixture. Slowly add 5% Na₂S-9H₂O solution to the medium, wait for the medium to become colorless, then adjust pH to 5.5 with H₂SO₄. Distribute medium into culture vessels under the same gas mixture, seal with butyl rubber stoppers, and autoclave. Separately autoclave 10% KNO₃ and 20% MES solutions (pH 5.5) under a N₂ atmosphere. Prior to inoculation, add the sterile KNO₃ and MES solutions, as well as an additional 3% Na₂S-9H₂O solution (neutralized, autoclaved under a N₂ atmosphere, 4 ml per liter of the medium) to the medium. Pressurize the inoculated bottles to 200kPa H₂-CO₂ (4:1, v/v) gas mixture.

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