

559. TMBS4 MEDIUM**Solution A:**

NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.15	g
KCl	0.50	g
NH ₄ Cl	0.25	g
KH ₂ PO ₄	0.20	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite/tungstate solution (see medium 385)	1.00	ml
Resazurin	0.50	mg
Distilled water	930.00	ml

Solution B:

NaHCO ₃ , 5% w/v solution	30.00	ml
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Solution C:

Vitamin solution (see next page)	10.00	ml
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Solution D:

Na-thiosulfate, 5% w/v solution	2.00	ml
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Solution E:

Yeast extract, 10% w/v solution	10.00	ml
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Solution F:

Syringate solution (see next page)	10.00	ml
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Solution G:

Dithiothreitol, 100 mM solution	10.00	ml
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Boil solution A for 3 min., then cool to room temperature under 100% N₂ gas atmosphere. Dispense the medium under same gas atmosphere in culture vessels and autoclave. Autoclave separately solutions E and F under 100% N₂ and solution B under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C, D, and G are prepared under N₂ gas atmosphere and sterilized by filtration.

Solutions B to G are added to the sterile, cooled solution A in the sequence as indicated. The final pH of the medium should be 7.2-7.4. After inoculation add 25 mg sodium dithionite per liter medium from a 2.5% (w/v) solution, freshly prepared under N₂ and filter-sterilized.

Vitamin solution and syringate solution: *see next page*

Vitamin solution:

Use vitamin solution of medium 141, but increase amount of vitamin B₁₂ to 5.0 mg/l and adjust final pH of solution to 7.2 using 50 mM sodium phosphate buffer.

Syringate solution:

Dissolve syringic acid in distilled water and neutralize solution with 2 N NaOH until a pH of 7.5 is reached. The final concentration of syringate in the solution should be 6% w/v.