

195d. KNH MEDIUM

Solution A:

Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
NaCl	21.00	g
MgCl ₂ x 6 H ₂ O	3.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Yeast extract	0.50	g
Ascorbic acid	0.10	g
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B:

Trace element solution SL-10 (see medium 320)	1.00	ml
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Solution C:

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution D:

Na-acetate	0.70	g
Distilled water	10.00	ml

Solution E:

Vitamin solution (see medium 141)	10.00	ml
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Solution F:

TRIS-HCl (1.0 M solution, pH 8.0)	7.20	ml
NaNTA (0.5 M solution)	4.80	ml
TiCl ₃ (15% w/v solution in HCl; Riedel-de Haen)	0.55	ml

Sterilize by filtration.

Solution A is sparged with 80% H₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. *Solutions B* and *D* are autoclaved separately under 100% N₂ gas. *Solution C* is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere.

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Solutions E and F are prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium *solutions B to F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4. After inoculation add 1 bar overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.