

169b: LEADBETTERA MEDIUM

Solution A	952.00	ml
Solution B	20.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Dissolve ingredients of solution A (except cysteine) and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere, solution C is autoclaved under 100% N₂ gas atmosphere and solutions D and E are sterilized by filtration under 100% N₂ gas atmosphere. To complete the medium appropriate amounts of solutions B, C, D and E are added to the sterile solution A in the sequence as indicated. Final pH of the complete medium should be 7.5.

Solution A

CaCl ₂ x 2 H ₂ O	0.04	g
MgSO ₄ x 7 H ₂ O	0.50	g
Yeast extract (OXOID)	4.00	g
D-Glucose	4.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	950.00	ml

Solution B

NaHCO ₃	1.00	g
Distilled water	20.00	ml

Solution C

K ₂ HPO ₄ (0.5 M)	16.04	ml
KH ₂ PO ₄ (0.5 M)	3.96	ml

Combine 16.04 ml of 0.5 M K₂HPO₄ with 3.96 ml of 0.5 M KH₂PO₄ stock solution to reach a pH of around 7.4.

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Solution D

Seven vitamins solution	1.00	ml
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Solution E

DL-Dithiothreitol	0.15	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml