

144: THERMOANAEROBACTER BROCKII MEDIUM

This recipe contains strain-specific modifications for *Halanaerobium acetethylicum* DSM 3532 *

Final pH: 7.2 - 7.4

Final volume: 1017 ml

NH ₄ Cl	0.90	g
NaCl	0.90	g
MgCl ₂ x 6 H ₂ O	0.40	g
KH ₂ PO ₄	0.75	g
K ₂ HPO ₄	1.50	g
Trace element solution	9.00	ml
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	3.00	ml
Yeast extract	3.00	g
Trypticase peptone (BD BBL)	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution	5.00	ml
D-Glucose	5.00	g
Na ₂ S x 9 H ₂ O	1.00	g
NaCl	100.00	g/l
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, glucose and sulfide), sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Adjust pH of complete medium to 7.2 - 7.4.

* Add 10% NaCl; anaerobic

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₂ x 4 H ₂ O	0.20	g
MnCl ₂ x 4 H ₂ O	0.10	g
CoCl ₂ x 6 H ₂ O	0.17	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnCl ₂	0.10	g
CuCl ₂	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
NaCl	1.00	g

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Na ₂ SeO ₃ x 5 H ₂ O	0.03	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

Wolin's vitamin solution (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml