

838: GEOANAEROBACTER MEDIUM

This recipe contains strain-specific modifications for *Desulfitobacterium hafniense* DSM 16228 *

Final pH: 6.8

Final volume: 1002 ml

Ferric citrate monohydrate	10.00	g
KH ₂ PO ₄	0.60	g
NH ₄ Cl	0.30	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.10	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Na ₂ CO ₃	1.50	g
Na-acetate	0.80	g
Na-ascorbate	0.80	g
Na-(DL)-lactate	1.00	g
Yeast extract	1.00	g
Wolin's vitamin solution	10.00	ml
Distilled water	1000.00	ml

First dissolve ferric citrate by heating the water under continuous stirring. After cooling to room temperature adjust the pH to 6.0, then add and dissolve the remaining ingredients, except carbonate, acetate and ascorbate. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add acetate and ascorbate from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to inoculation check the medium pH and adjust to 6.8, if necessary.

* Omit Na-acetate and Na-ascorbate. Supplement medium after autoclaving with 10.00 ml/l of a Wolin's vitamin solution (see medium 141), 1.00 g/l Na-DL-lactate and 1.00 g/l yeast extract added from sterile anoxic stock solutions prepared under 100% N₂ gas.

Trace element solution SL-11 (from medium 722)

Na ₂ -EDTA x 2 H ₂ O	5.20	g
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

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NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in 800 ml distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds. Finally adjust pH to 6.0 and bring volume to 1000 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

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