

1042: VULCANIBACILLUS MEDIUM

This recipe contains strain-specific modifications for *Tepidibacillus fermentans* DSM 23802 *

Final pH: 6.8 - 7.0

Final volume: 1005 ml

Sea Salt (SIGMA)	30.00	g
NaNO ₃	1.00	g
Wolfe's mineral elixir	2.00	ml
VOSO ₄ x 5 H ₂ O (0.01% w/v)	0.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
FeSO ₄ x 7 H ₂ O	0.10	g
NaHCO ₃	1.00	g
Yeast extract	0.50	g
Na pyruvate	1.00	g
Wolin's vitamin solution (10x)	2.00	ml
D-Glucose	2.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins), adjust pH to 7.0 and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense the medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) FeSO₄ x 7 H₂O in 0.1 N H₂SO₄. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0.

2. Note: It may be necessary to add 10-20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under 100% N₂ and filter-sterilized), if the medium is not completely reduced after inoculation.

* Replace pyruvate with 2.50 g/l D-glucose added to the autoclaved medium from an anoxic sterile stock solution.

Wolfe's mineral elixir (from medium 792)

MgSO ₄ x 7 H ₂ O	30.00	g
MnSO ₄ x H ₂ O	5.00	g
NaCl	10.00	g
FeSO ₄ x 7 H ₂ O	1.00	g
CoCl ₂ x 6 H ₂ O	1.80	g
CaCl ₂ x 2 H ₂ O	1.00	g
ZnSO ₄ x 7 H ₂ O	1.80	g

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$\text{CuSO}_4 \times 5 \text{ H}_2\text{O}$	0.10	g
$\text{AlK}(\text{SO}_4)_2 \times 12 \text{ H}_2\text{O}$	0.18	g
H_3BO_3	0.10	g
$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$	0.10	g
$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \times 6 \text{ H}_2\text{O}$	2.80	g
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	0.10	g
Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4 , then add and dissolve the salts.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml