

ATCC Medium 0376: Sludge-Free Methanobacterium Medium (DSM 1523)

KH ₂ PO ₄	0.50 g
MgSO ₄ x 7 H ₂ O	0.40 g
NaCl	0.40 g
NH ₄ Cl	0.40 g
CaCl ₂ x 2 H ₂ O	0.05 g
Trace element solution SL-10	1.00 ml
Brain heart infusion (BD Bacto)	6.00 g
Proteose peptone (BD Difco)	6.00 g
Yeast extract (OXOID)	2.00 g
Na-acetate	1.00 g
Na-formate	2.00 g
Sodium resazurin (0.1% w/v)	0.50 ml
NaHCO ₃	4.00 g
Seven vitamins solution	1.00 ml
L-Cysteine HCl x H ₂ O	0.50 g
Na ₂ S x 9 H ₂ O (Sodium Sulfide Nonahydrate)	0.50 g
Distilled water	1000.00 ml

Dissolve ingredients except vitamins, cysteine, and Sodium sulfide

Adjust pH to 6.5.

Dispense medium Hungate-type tubes or serum vials (or as requested) to 30% of their volume (as requested)

Autoclave

Add vitamins solution, cysteine, and sodium sulfide from sterile stock solutions (filter sterilize Vitamin solutions)

Before use, check the complete medium's pH and adjust it to 6.8 - 7.0, if necessary.

Trace element solution SL-10

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.

Seven vitamins solution

Vitamin B12	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	200.00 mg
Distilled water	1000.00 ml