

507: CARBOXYDOTHERMUS MEDIUM

This recipe contains strain-specific modifications for *Carboxydothemus pertinax* DSM 23698 *

Final pH: * 6.2

Final volume: 1022 ml

KCl	0.33	g
MgCl ₂ x 6 H ₂ O	0.52	g
CaCl ₂ x 2 H ₂ O	0.29	g
NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Yeast extract	0.05	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	20.00	ml
Na-pyruvate	2.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, yeast extract, vitamins and sulfide, then 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 to 30% of their volume and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Neutralize the sodium sulfide with sterile H₂SO₄ (see medium 29). After completing the medium adjust pH to 6.8-7.0.

2. Inoculated vessels are pressurized with sterile carbon monoxide gas to 2 bar overpressure.

* Supplement medium with 2.00 g/l Na-pyruvate and omit sulfide. Adjust final pH of medium to 6.2. Change gas phase to 100% carbon monoxide prior to inoculation, but do not use excess pressure.

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 × 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ × 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.

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

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na₂S × 9 H₂O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H₂SO₄ drop-wise with a syringe without opening the bottle.