

465: MINERAL MEDIUM PH 7.25

This recipe contains strain-specific modifications for *Cupriavidus necator* DSM 11098 *

Final pH: 7.2

Final volume: 1000 ml

Na ₂ HPO ₄ x 2 H ₂ O	3.50	g
KH ₂ PO ₄	1.00	g
(NH ₄) ₂ SO ₄	0.50	g
MgCl ₂ x 6 H ₂ O	0.10	g
Ca(NO ₃) ₂ x 4 H ₂ O	0.05	g
Trace element solution SL-4	1.00	ml
2-hydroxymethyl-tetrahydrofuran	3.00	ml/l
Distilled water	1000.00	ml

1. pH 7.25

2. Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220, 535 or 830). After this reactivation, cultivate on mineral medium with the appropriate carbon source.

* With 3ml/l 2-hydroxymethyl-tetrahydrofuran

Main sol. 1 (from medium 1)

Peptone	5.00	g
Meat extract	3.00	g
Agar, for solid medium	15.00	g
Distilled water	1000.00	ml

1. Adjust pH to 7.0.

2. For Bacillus strains the addition of 10.0 mg MnSO₄ x H₂O is recommended for sporulation.

Main sol. 220 (from medium 220)

Casein peptone	15.00	g
Soy peptone	5.00	g
NaCl	5.00	g
Agar	15.00	g
Distilled water	1000.00	ml

Adjust pH to 7.3. Medium is identical with Tryptone Soya Agar (Oxoid CM 131).

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Main sol. 535 (from medium 535)

Trypticase soy broth (BBL 11768, Oxoid CM129 or Merck 30469)	30.00	g
Agar	20.00	g
Distilled water	1000.00	ml

1. pH 7.3
2. Autoclave at 121°C for 15 min.

Main sol. 830 (from medium 830)

Yeast extract	0.50	g
Proteose peptone (Difco no. 3)	0.50	g
Casamino acids	0.50	g
Glucose	0.50	g
Starch (soluble)	0.50	g
Na-pyruvate	0.30	g
K ₂ HPO ₄	0.30	g
MgSO ₄ x 7 H ₂ O	0.05	g
Agar	15.00	g
Distilled water	1000.00	ml

Final pH 7.2; adjust with crystalline K₂HPO₄ or KH₂PO₄ before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.

Trace element solution SL-4 (from medium 14)

Na ₂ -EDTA	0.50	g
FeSO ₄ x 7 H ₂ O	0.20	g
ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.30	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.02	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
Distilled water	1000.00	ml

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other compounds.