

504: ANAEROBIC SEAWATER (SWM) MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and D are prepared under 100% N₂ gas and sterilized by filtration. Solution E is autoclaved under 100% N₂ gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.2 – 7.4, if necessary.

For DSM 5848, DSM 9537: Replace glucose with 2.50 g/l of Na₂-succinate and 0.50 g/l yeast extract as substrates.

For DSM 6233: Replace glucose with 0.50 g/l of pyrogallol as the substrate. When growth appears feed once more with 0.50 g/l pyrogallol.

For DSM 12881, DSM 19012, DSM 25728: Supplement medium with 0.50 g/l yeast extract.

For DSM 15285, DSM 19306: Replace glucose with 10.00 g/l Proteose peptone (BD Difco) as substrate. Adjust pH of complete medium to 6.5 - 7.0.

For DSM 19335, DSM 17953: Replace glucose with 2.50 g/l maltose and 2.00 g/l yeast extract as substrates.

For DSM 106009: Use 0.30 g/l L-Cysteine-HCl x H₂O and 1.50 g/l acetaldehyde as substrates. Acetaldehyde should be added in aliquots of 0.20 g/l at regular intervals until full growth is achieved.

Solution A

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	20.00	g
MgCl ₂ x 6 H ₂ O	3.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

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Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

D-Glucose	2.00	g
Distilled water	20.00	ml

Solution D

Seven vitamins solution	1.00	ml
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Solution E

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

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 (from medium 503)

Vitamin B ₁₂	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 x 2 H ₂ O	200.00	mg
Distilled water	1000.00	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