

503. ANAEROBIC FRESHWATER (FWM) MEDIUM

Solution A:

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Solution B:

Trace element solution SL-10 (see medium 320)	1.00	ml
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Solution C:

Vitamins solution (see below)	1.00	ml
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Solution D:

Selenite-tungstate solution (see medium 385)	1.00	ml
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Solution E:

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution F:

Substrate solution (see below)

Solution G:

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	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. *Solutions B, D and G* are autoclaved separately under 100% N₂ gas. *Solutions C and F* are prepared under 100% N₂ gas and sterilized by filtration. *Solution E* is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. To complete the medium appropriate amounts of *solutions B to G* are added to the sterile *solution A* in the sequence as indicated. Adjust pH of complete medium to 7.2 - 7.4, if necessary.

Substrates and additional instructions: see next pages!

Seven vitamins solution:

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

For DSM 5847:

Use 1.50 g/l of Na-(D/L)-3-hydroxybutyrate or 2.00 g/l D-fructose as substrate.

For DSM 5849:

Use 2.50 g/l of Na₂-succinate as substrate.

For DSM 5885:

Use 1.60 g/l Na₂-maleate as substrate. Adjust pH of complete medium to 6.7 - 6.8.

For DSM 6779:

Use 2.00 g/l of xylan or xylose as substrate.

For DSM 10092:

Add 0.70 g/l Na₂SO₄ to *solution A* and use 1.50 g/l Na-propionate as substrate. Reduce the amount of sulfide to 0.10 g/l and use 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) for reduction of the medium prior to inoculation.

For DSM 11045:

Use as final concentration: 10 mM taurine, 60 mM Na-formate, and 200 µM 1,4-naphthochinone as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 11046:

Add 1.70 g/l NaNO₃ to *solution A* and use 1.25 g/l taurine as substrate.

For DSM 11261:

Use 1.00 g/l yeast extract and 2.00 g/l Na-glycolate as substrates.

For DSM 11262:

Use 1.00 g/l yeast extract and 2.50 g/l Na-(D/L)-3-hydroxybutyrate as substrates.

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For DSM 11263 and DSM 11489:

Use 0.82 g/l Na-acetate and 3.20 g/l Na₂-fumarate as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 11270:

Use 2.50 g/l taurine as substrate.

For DSM 11480 and DSM 12595:

Use 1.80 g/l D-glucose as substrate added from an anoxic stock solution sterilized by filtration.

For DSM 12018:

Use 0.20 g/l yeast extract and 2.66 g/l L-aspartic acid as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 13305:

Use 1.00 g/l yeast extract and 5.00 g/l D-fructose as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 14424, DSM 28450, DSM 100378, DSM 103557 and DSM 105239:

Use 2.00 g/l yeast extract and 5.00 g/l D-glucose as substrates added from anoxic stock solutions sterilized by filtration.

For DSM 15206:

Use 2.00 g/l yeast extract and 2.00 g/l Trypticase peptone (BD BBL) as substrates.

For DSM 15978 and DSM 27305:

Use 2.00 g/l trimethylamine hydrochloride and 0.60 g/l methanol as substrates and adjust pH of the medium to 6.5 - 7.0. After inoculation add 10.00 ml/l of a sterile anoxic stock solution of FeSO₄ x 7 H₂O (0.2% w/v).

For DSM 21661, DSM 21662 and DSM 21683:

Replace *solutions B* and *D* with the trace elements solution of medium 141, *solution C* with the vitamins solution of medium 141 and omit *solution G*. Use 1.36 g/l Na-acetate as substrate. Prior to inoculation 1.22 g/l Na-perchlorate is added from a sterile anoxic stock solution sterilized by filtration.

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For DSM 24856:

Add 0.85 g/l NaNO₃ to *solution A*. Use 0.10 g/l yeast extract and 1.10 g/l Na-pyruvate as substrates. Na-pyruvate is added to the autoclaved medium from a sterile anoxic stock solution sterilized by filtration.

For DSM 25964:

Use 0.50 g/l Na-pyruvate as substrate added from an anoxic stock solution sterilized by filtration.

For DSM 27520:

Omit sodium carbonate and use 1.00 g/l yeast extract and 2.00 g/l D-glucose as substrates added from anoxic stock solutions sterilized by filtration. Adjust pH of complete medium to 5.5, if necessary.

For DSM 103526:

Use 1.00 g/l yeast extract and 0.40 g/l D-glucose as substrates added from anoxic stock solutions sterilized by filtration.