

## 503. ANAEROBIC FRESHWATER (FWM) MEDIUM

### Solution A:

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> 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:

NaHCO <sub>3</sub>	2.50	g
Distilled water	50.00	ml

### Solution F:

Substrate solution (see below)		
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### Solution G:

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	10.00	ml

Sparge *solution A* with 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas. *Solutions C* and *F* are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. *Solution E* is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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 <sub>12</sub>	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 <sub>2</sub> 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 Na<sub>2</sub>-succinate as substrate.

For DSM 5885:

Use 1.60 g/l Na<sub>2</sub>-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 Na<sub>2</sub>SO<sub>4</sub> 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 N<sub>2</sub> and filter-sterilized) for reduction of the medium prior to inoculation.

For DSM 11046:

Add 1.70 g/l NaNO<sub>3</sub> 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 Na<sub>2</sub>-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:

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 FeSO<sub>4</sub> x 7 H<sub>2</sub>O (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 NaNO<sub>3</sub> 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 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.