

## 503: ANAEROBIC FRESHWATER (FWM) MEDIUM

Final pH: 7.2 - 7.4

Final volume: 1003 ml

<b>Solution A</b>	942.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	20.00	ml
<b>Solution D</b>	1.00	ml
<b>Solution E</b>	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. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solution C and D are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Solution E is autoclaved under 100% N<sub>2</sub> 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 5847: Replace D-glucose with 1.50 g/l of Na (D/L) 3 hydroxybutyrate or 2.00 g/l D-fructose as substrate.

For DSM 5849: Replace D-glucose with 2.50 g/l of Na<sub>2</sub> succinate as substrate.

For DSM 5885: Replace D-glucose with 1.60 g/l Na<sub>2</sub>-maleate as substrate added from an anoxic stock solution sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8.

For DSM 6779: Replace D-glucose with 2.00 g/l of xylose or xylan as substrate.

For DSM 10092: Omit glucose. 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 25 - 50 mg sodium dithionite per liter (e.g. from a freshly prepared filter-sterilized 5% w/v solution) for reduction of the medium prior to inoculation.

For DSM 11045: Omit solution C. 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 NaNO<sub>3</sub> to solution A and replace D-glucose with 1.25 g/l taurine as substrate.

For DSM 11261: Replace D-glucose with 1.00 g/l yeast extract and 2.00 g/l Na glycolate as substrates.

For DSM 11262: Replace D-glucose with 1.00 g/l yeast extract and 2.50 g/l Na (D/L)-3 hydroxybutyrate as substrates.

For DSM 11263, DSM 11489: Replace D-glucose with 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.

## 503: ANAEROBIC FRESHWATER (FWM) MEDIUM

For DSM 11270: Replace D-glucose with 2.50 g/l taurine as substrate.

For DSM 11480, DSM 12595: Reduce amount of D-glucose to 1.80 g/l.

For DSM 12018: Replace D-glucose with 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: Replace D-glucose with 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, DSM 105239: Supplement medium with 2.00 g/l yeast extract added from an anoxic stock solution sterilized by filtration.

For DSM 15206: Replace D-glucose with 2.00 g/l yeast extract and 2.00 g/l Trypticase peptone (BD BBL) as substrates.

For DSM 15978, DSM 27305: Supplement medium with 20 ml/l  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  solution (0.1% w/v), 1 ml/l Na-dithionite solution (5% w/v), 0.8 ml/l Methanol, and 2 g/l Trimethylamine-HCl. Omit D-Glucose.

For DSM 19598: Reduce amount of glucose to 2 mM (0.36 g/l); defined coculture with *Methanospirillum hungatei*. Incubate in the dark. Anaerobic

For DSM 24856: Omit glucose. Add 0.85 g/l  $\text{NaNO}_3$  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: Replace D-glucose with 0.50 g/l Na-pyruvate as substrate added from an anoxic stock solution sterilized by filtration. Supplement solution A with 2.0 g/l yeast extract.

For DSM 27520: Omit sodium carbonate, reduce amount of D-glucose to 2.00 g/l and add 1.00 g/l yeast extract as substrate. Adjust pH of complete medium to 5.5.

For DSM 27582: Supplement medium after autoclaving with 0.1 g/l yeast extract, 2.0 g/l D-mannose, and 1 ml/l Wolin's vitamin solution (10x) added from anoxic stock solutions sterilized by filtration. Omit D-Glucose.

For DSM 100618: Reduce glucose to 3g/l. Add 1g/l yeast extract.

For DSM 101182: Supplement medium after autoclaving with 0.50 g/l yeast extract and 0.40 g/l L-cysteine HCl  $\times \text{H}_2\text{O}$  added from sterile anoxic stock solutions.

For DSM 103526: Reduce amount of D-glucose to 0.40 g/l and add 1.00 g/l yeast extract as substrate.

For DSM 111565: Replace D-glucose with 0.50 g/l yeast extract and 1.00 g/l L-rhamnose as substrates added from anoxic stock solutions sterilized by filtration .

For DSM 112739: Omit glucose and sodium resazurin. Supplement medium with 2.20 g/l sodium phosphite hydrate.

## 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
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

### Solution B

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Distilled water	30.00	ml

### Solution C

D-Glucose	5.00	g
Distilled water	20.00	ml

### Solution D

<b>Seven vitamins solution</b>	1.00	ml
--------------------------------	------	----

### Solution E

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

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

## 503: ANAEROBIC FRESHWATER (FWM) MEDIUM

### **Selenite-tungstate solution** (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### **Seven vitamins solution** (from medium 503)

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