

## 383b. DESULFONAUTICUS MEDIUM

### Solution A:

Na <sub>2</sub> SO <sub>4</sub>	3.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.30	g
NaCl	21.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	3.00	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

### Solution B:

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

Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Distilled water	20.00	ml

### Solution D:

Na-acetate	1.00	g
Distilled water	10.00	ml

### Solution E:

Vitamin solution (see medium 141)	10.00	ml
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### Solution F:

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	13.00	ml

*Solution A* is sparged with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then dispensed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. *Solutions B, D* and *F* are autoclaved separately under 100% N<sub>2</sub> gas. *Solution C* is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. *Solution E* is prepared under 100% N<sub>2</sub> gas and filter-sterilized. To complete the medium appropriate amounts of *solutions B* to *F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 7.0 – 7.2. After inoculation pressurize vials to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

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*Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum. Incubate all strains in the dark.*

For DSM 3382 omit Na-acetate.

For DSM 4206 and DSM 15269 supplement medium with 2.00 g/l Trypticase peptone and 2.00 g/l yeast extract added to the autoclaved medium from sterile anoxic stock solutions prepared under N<sub>2</sub> gas.

For DSM 15286, DSM 21156 and DSM 101864 supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added to the autoclaved medium from an anoxic stock solution sterilized by filtration. Adjust pH of medium to **6.5 – 6.7** prior to inoculation.