

## **ATCC Medium: 2760 MOPS Buffered Medium for Dehalococcoides sp.**

### **Basal Medium**

Salt solution (See Below).....	20 ml
Trace element solution (See Below).....	1 ml
Se/Wo solution (See Below).....	1 ml
Resazurin solution (See Below).....	0.25 ml
Sodium acetate.....	0.41 g
DI Water.....	956.5 ml

Boil and cool down to room temperature under N<sub>2</sub>, then add the following components:

Na <sub>2</sub> S.....	0.2 mmol
L-cysteine.....	0.2 mmol

Autoclave medium at 121°C and after the medium has cooled to room temperature add the following stock solutions:

NaHCO <sub>3</sub> (See Below).....	1 ml
MOPS (See Below).....	20 ml
Ti(III)NTA(See Below).....	20 ml
3-vitamin solution(See Below).....	0.5 ml
6-vitamin solution (See Below).....	1 ml
Vitamin B12 solution (See Below).....	1 ml

Dispense anaerobically under 100% nitrogen into appropriate vessel.

### **Salt solution**

NaCl.....	50.0 g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	20.5 g
NH <sub>4</sub> Cl.....	13.5 g
KCl.....	26.0 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.75 g
KH <sub>2</sub> PO <sub>4</sub> .....	10.0 g
DI Water.....	1000 ml

Store at room temperature.

### **Trace element solution**

HCl (25% w/w).....	10 ml
FeCl <sub>2</sub> ·4H <sub>2</sub> O.....	1.5 g
H <sub>3</sub> BO <sub>3</sub> .....	6.0 mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0 mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0 mg
ZnCl <sub>2</sub> .....	70.0 mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	36.0 mg

NiCl<sub>2</sub>·6H<sub>2</sub>O.....24.0 mg  
CuCl<sub>2</sub>·2H<sub>2</sub>O.....2.0 mg  
DI Water.....1000 ml  
Store at room temperature in the dark.

**Se/Wo solution**

NaOH.....0.5 g  
Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O.....6.0 mg  
Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O.....8.0 mg  
DI Water.....1000 ml  
Store at room temperature in the dark.

**Resazurin solution**

Resazurin.....0.1 g  
DI Water.....100 ml

**Stock solutions (add aseptically)**

NaHCO<sub>3</sub> 1M autoclaved  
MOPS, 1M pH adjusted to 7.45, filter sterilized  
Ti(III)NTA, 25mM, filter sterilized

**3-vitamin solution**

Folic acid.....1.0 mg  
Riboflavin.....2.5 mg  
DL-6,8-thioctic.....2.5 mg  
DI Water.....250.0 ml  
Filter sterilize: store at 4°C in the dark.

**6-vitamin solution**

Biotin.....1.0 mg  
4-aminobenzoic acid.....5.0 mg  
Pantothenate.....1.0 mg  
Pyridoxamine.....25.0 mg  
Nicotinic acid.....10.0 mg  
Thiamine.....2.0 mg  
DI Water.....100.0 ml  
Filter sterilize: store at 4°C in the dark.

**Vitamin B12 solution**

Vitamin B12.....2.5 mg  
DI Water.....100.0 ml  
Filter sterilize: store at 4°C in the dark.