

## 732: DEHALOBACTER RESTRICTUS MEDIUM (TCE)

<b>Solution A</b>	870.00	ml
<b>Solution B</b>	100.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	1.00	ml
<b>Solution E</b>	2.00	ml
<b>Solution F</b>	10.00	ml
<b>Solution G</b>	15.00	ml

1. Sparge solution A with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., 9 ml medium in 50 ml bottle). Pressurize closed bottles with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 0.5 bar overpressure and autoclave. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C, D and F are autoclave under 100% N<sub>2</sub> gas atmosphere. Solution E is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Prepare solution G by filling 13.5 ml hexadecane into a 50 ml serum bottle, then sparge with 100% N<sub>2</sub> gas to make it anoxic and autoclave. Add 1.50 ml anoxic autoclaved tetrachloroethene to the sterile anoxic hexadecane solution by syringe. To complete the medium add appropriate amounts of solutions B to F to the sterile solution A in the sequence as indicated. The pH of the medium before inoculation should be at 7.2.

2. Add solution G only after inoculation of the medium!

### Solution A

K <sub>2</sub> HPO <sub>4</sub>	0.65	g
NaH <sub>2</sub> PO <sub>4</sub> x H <sub>2</sub> O	0.17	g
Peptone (BD Bacto)	0.10	g
Na-acetate	0.46	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	870.00	ml

### Solution B

(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.27	g
NaHCO <sub>3</sub>	3.73	g
Distilled water	100.00	ml

### Solution C

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
Distilled water	10.00	ml

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### Solution D

Trace element solution	1.00	ml
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### Solution E

Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml

### Solution F

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

### Solution G

Hexadecane	13.50	ml
Tetrachloroethene	1.50	ml

### Trace element solution (from medium 732)

Na <sub>2</sub> -EDTA	0.50	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	2.00	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
AlCl <sub>3</sub>	10.00	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	1000.00	ml

First dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add remaining compounds.

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg

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Distilled water	1000.00	ml
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### 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