

732. DEHALOBACTER RESTRICTUS MEDIUM (TCE)**Solution A:**

K ₂ HPO ₄	0.65	g
NaH ₂ PO ₄ x H ₂ O	0.17	g
Peptone (BD Bacto)	0.10	g
Na-acetate	0.46	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	900.00	ml

Solution B:

(NH ₄) ₂ CO ₃	0.27	g
NaHCO ₃	3.73	g
Distilled water	100.00	ml

Solution C:

CaCl ₂ x 2 H ₂ O	0.11	g
MgCl ₂ x 6 H ₂ O	0.10	g
Distilled water	10.00	ml

Solution D:

Trace element solution (see below)	1.00	ml
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Solution E:

Vitamin solution of medium 141	9.00	ml
Vitamin solution of medium 503	1.00	ml

Solution F:

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Solution G:

Hexadecane	13.50	ml
Tetrachloroethene	1.50	ml

Sparge *solution A* with 80% H₂ and 20% CO₂ 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% H₂ and 20% CO₂ gas mixture to 0.5 bar overpressure and autoclave. *Solution B* is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. *Solutions C, D* and *F* are autoclave under 100% N₂ gas atmosphere.

Continued on next page

Solution E is prepared under 100% N₂ 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% N₂ 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. Add solution G only after inoculation of the medium!

Trace element solution:

Na ₂ -EDTA	0.50 g
FeCl ₂ x 4 H ₂ O	2.00 g
ZnCl ₂	70.00 mg
MnCl ₂ x 4 H ₂ O	100.00 mg
H ₃ BO ₃	6.00 mg
CoCl ₂ x 6 H ₂ O	190.00 mg
CuCl ₂ x 2 H ₂ O	2.00 mg
AlCl ₃	10.00 mg
NiCl ₂ x 6 H ₂ O	24.00 mg
Na ₂ MoO ₄ x 2 H ₂ 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.