

732a: DESULFUROMONAS MEDIUM (TCE)

This recipe contains strain-specific modifications for *Sulfurospirillum halorespirans* DSM 13726 *

Final pH: 7.2

Final volume: 1008 ml

Solution A	870.00	ml
Solution B	100.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	2.00	ml
Solution F	10.00	ml
Solution G	15.00	ml

1. Sparge solution A with 80% N₂ 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 in 50 ml bottles) 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. 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.

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

* Omit acetate from solution A and add 2.50 g/l Na-DL-lactate to the medium from a sterile anoxic stock solution prepared under 100% N₂ gas.

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
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na-DL-lactate	2.50	g
Distilled water	870.00	ml

Solution B

(NH ₄) ₂ CO ₃	0.27	g
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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	1.00	ml
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Solution E

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

Selenite-tungstate solution (from medium 385)

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

Trace element solution (from medium 732)

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

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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.

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 ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	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 ₂ O	200.00	mg
Distilled water	1000.00	ml