

732a: DESULFUROMONAS MEDIUM (TCE)

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!

For DSM 13726: 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
Distilled water	870.00	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

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Distilled water	10.00	ml
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Solution D

Trace element solution	1.00	ml
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Solution E

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

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

Hexadecane	13.50	ml
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Tetrachloroethene	1.50	ml
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Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
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Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
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Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
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Distilled water	1000.00	ml
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Trace element solution (from medium 732)

Na ₂ -EDTA	0.50	g
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FeCl ₂ x 4 H ₂ O	2.00	g
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ZnCl ₂	70.00	mg
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MnCl ₂ x 4 H ₂ O	100.00	mg
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H ₃ BO ₃	6.00	mg
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CoCl ₂ x 6 H ₂ O	190.00	mg
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CuCl ₂ x 2 H ₂ O	2.00	mg
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AlCl ₃	10.00	mg
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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
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Distilled water	1000.00	ml
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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.

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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