

497: CLOSTRIDIUM KN MEDIUM (HYDROXYBENZOATE)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and D are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solution E is autoclaved under 100% N₂ gas atmosphere. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. Note: Some cultures are shipped in semi-solid medium which stimulates growth at the beginning. For agar stabs 3.00 g/l agar are added to the complete medium from a sterile anoxic stock solution (2% w/v). Upon receipt add anoxically 1 - 2 ml of the recommended freshly prepared liquid medium to the agar tube and incubate for 3 - 5 days. After incubation transfer 0.5 ml of the resulting cell suspension in the liquid phase to tubes with liquid medium.

For DSM 5672: Replace 2,4-dihydroxybenzoic acid with 0.15 g/l salicylic acid (2-hydroxybenzoic acid). The concentration of salicylic acid in the medium should not exceed 1.5 mmol per liter; feed substrate several times in order to reach a reasonable cell density.

For DSM 5673: Replace 2,4-dihydroxybenzoic acid with 0.75 g/l 3-hydroxybenzoic acid.

Solution A

Na ₂ SO ₄	1.50	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

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Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml
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Solution D

2,4-Dihydroxybenzoic acid	0.40	g
Distilled water	10.00	ml

Solution E

Na ₂ S x 9 H ₂ O	0.40	g
Distilled water	10.00	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 SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	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
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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

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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