

497. CLOSTRIDIUM KN MEDIUM (HYDROXYBENZOATE)**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
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	920.00	ml

Solution B:

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C:

Trace element solution SL-10 (see medium 320)	1.00	ml
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Solution D:

Vitamin solution (see medium 141)	10.00	ml
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Solution E:

Substrate (see below)

Solution F:

Na ₂ S x 9 H ₂ O	0.40	g
Distilled water	10.00	ml

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 *F* are autoclaved under 100% N₂ gas atmosphere. *Solutions D* and *E* are prepared under 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of *solutions B* to *F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

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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.

Solution E and additional instructions: see below!

DSM 5671:

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

DSM 5672:

2-Hydroxybenzoic acid	0.15	g
Distilled water	10.00	ml

The concentration of 2-hydroxybenzoate in the medium should not exceed 1.5 mmol per liter; feed substrate several times in order to reach a reasonable cell density.

DSM 5673:

3-Hydroxybenzoic acid	0.75	g
Distilled water	10.00	ml