

523: CLOSTRIDIUM CHARTATABIDUM MEDIUM

Mineral solution	38.00	ml
Clarified rumen fluid	200.00	ml
K ₂ HPO ₄	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	0.50	g
Haemin solution (0.05% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	4.00	g
Cellobiose	5.00	g
L-Cysteine HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	760.00	ml

Dissolve ingredients (except carbonate, cellobiose, cysteine and sulfide), bring medium to the boil, then cool to room temperature under 100% CO₂ gas atmosphere. Add the carbonate and equilibrate the medium with the CO₂ gas to pH 6.8. Distribute under CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add cellobiose, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.

Mineral solution (from medium 330)

KH ₂ PO ₄	6.00	g
NaCl	12.00	g
(NH ₄) ₂ SO ₄	6.00	g
CaCl ₂ x 2 H ₂ O	1.60	g
MgSO ₄ x 7 H ₂ O	2.50	g
Distilled water	1000.00	ml

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml



523: CLOSTRIDIUM CHARTATABIDUM MEDIUM

Distilled water	100.00	ml
-----------------	--------	----

Dissolve 50 mg haemin in 1 ml 1 N NaOH; make up to 100 ml with distilled water and filter sterilize. Store refrigerated.