

**523. CLOSTRIDIUM CHARTATABIDUM MEDIUM**

Mineral solution (see medium 330)	38.00	ml
Rumen fluid, clarified (see medium 1310)	200.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.30	g
Trypticase peptone (BD BBL)	2.00	g
Yeast extract (OXOID)	0.50	g
Haemin solution (0.05% w/v, see medium 78)	2.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	4.00	g
Cellobiose	5.00	g
L-Cysteine-HCl x H <sub>2</sub> O	0.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> 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% CO<sub>2</sub> gas atmosphere. Add the carbonate and equilibrate the medium with the CO<sub>2</sub> gas to pH 6.8. Distribute under CO<sub>2</sub> 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% N<sub>2</sub> gas. Cellobiose has to be sterilized by filtration. Adjust pH of complete medium to 6.7 - 6.8, if necessary.