110a: CMC MEDIUM (N2/CO2)

Final pH: 7.0
Final volume: 1010 ml

Casitone	30.00	g
Yeast extract	5.00	g
K_2HPO_4	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Sugar mix	10.00	ml
Na_2CO_3	1.50	g
Meat filtrate	1000.00	ml

Dissolve ingredients, except cysteine, sugar mix, and carbonate, boil medium for 1 min, then cool to room temperature under 80% N_2 and 20% CO_2 gas atmosphere, add 0.5 g/l L-cysteine-HCl x H_2O and dispense under same gas atmosphere into Hungate-type tubes (for strains demanding meat particles put those first into the tube, use 1 part meat particles to 4 or 5 parts fluid). Autoclave at 121°C for 20 min. After autoclaving add the sugar mix from a sterile anoxic stock solution prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust the pH of the medium to pH 7.0, if necessary.

Meat filtrate

Ground beef (fat free)	500.00	g
NaOH (1 N)	25.00	ml
Distilled water	975.00	ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min with stirring. Cool to room temperature, skim fat off surface, and filter, retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1000.0 ml.

Sugar mix

D-Glucose	4.00	g
Cellobiose	1.00	g
D-Maltose	1.00	g
Starch (soluble)	1.00	g
Distilled water	10.00	ml