78c. CHOPPED MEAT MEDIUM FOR FERTIBACTERIUM SP.

Ground beef (fat free)	500.0	g
NaOH 1 N	25.0	ml
Casitone peptone (BD BACTO)	30.0	g
Yeast extract	5.0	g
K_2HPO_4	5.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml
L-Cysteine-HCl x H2O	0.5	g
Na-fumarate	6.0	g
Na-formate	6.0	g
Rumen fluid, clarified (see medium 1310)	50.0	ml
Horse serum, inactivated (INVITROGEN)	50.0	ml
Fatty acids mixture (see medium 119)	20.0	ml
Vitamin solution (see medium 141)	10.0	ml
Haemin solution (0.05% w/v, see medium 78)	5.0	ml
Vitamin K_3 solution (0.005% w/v, see medium 78)	5.0	ml
DL-Dithiothreitol (DTT)	0.3	g
Distilled water	700.0	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 Casitone, yeast extract, hydrogen phosphate and resazurin, then add water to a final volume of 700 ml. Sparge medium with 100% N_2 gas for 30 – 45 min to make it anoxic, then add cysteine and adjust pH to 7.0. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes containing meat particles (use ca. 7 ml liquid medium for 1 - 2 g meat particles), then autoclave at 121°C for 20 min. After autoclaving add fumarate, formate, rumen fluid, horse serum, fatty acids, vitamins, haemin, vitamin K_3 and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and sterilized by filtration.
For agar slants use 15 g agar per 1000.0 ml medium.