78b. CHOPPED MEAT MEDIUM (N2/CO2)

Ground beef (fat free)	500.0	g
Distilled water	1000.0	ml
NaOH 1 N	25.0	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 water to a final volume of 1000 ml, and then add:

Casitone	30.0	g
Yeast extract	5.0	g
K_2HPO_4	5.0	g
Na-resazurin solution (0.1% w/v)	0.5	ml

To make medium anoxic bring it to a boil, cool under 80% N_2 and 20% CO_2 gas mixture and add 0.5 g/l L-cysteine hydrochloride. Dispense under 80% N_2 and 20% CO_2 gas atmosphere by filling 7 ml medium into anoxic 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 adjust pH of complete medium to 7.0 using a sterile anoxic stock solution of Na_2CO_3 (5% w/v) prepared under 80% N_2 and 20% CO_2 gas mixture.

In some cases (as indicated in the catalogue) the addition of Haemin and Vitamin K_1 or Vitamin K_3 is necessary. Add to 1000 ml of medium after autoclaving:

Haemin solution (see below)	10.00	ml
Vitamin K_1 or Vitamin K_3 solution (see below)	10.00	ml

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

Vitamin K_1 solution:
Dissolve 0.1 ml of vitamin K_1 in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K_3 solution:
Dissolve 5 mg/ml of vitamin K_3 in 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.