

78b: CHOPPED MEAT MEDIUM (N₂/CO₂)

Ground beef (fat free)	500.00	g
NaOH (1 N)	25.00	ml
Casitone	30.00	g
Yeast extract	5.00	g
K ₂ HPO ₄	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	1000.00	ml

1. 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 80% N₂ and 20% CO₂ gas mixture 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.

2. In some cases (as indicated in the catalogue) the addition of Haemin and Vitamin K₁ or Vitamin K₃ is necessary. Add to 1000 ml of medium after autoclaving:

Haemin solution (optional)	10.00	ml
Vitamin K₁ solution (optional)	10.00	ml
Vitamin K₃ solution (optional)	1.00	ml

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml
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.

Vitamin K₁ solution (from medium 78)

Vitamin K ₁	0.10	ml
Ethanol (95 %)	20.00	ml

Dissolve 0.1 ml of vitamin K₁ in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.



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Vitamin K₃ solution

Vitamin K ₃	5.00	mg
Ethanol (95%)	1.00	ml
Water	100.00	ml

Dissolve 5 mg vitamin K₃ in 1 ml 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.