

110: CHOPPED MEAT MEDIUM WITH CARBOHYDRATES

This recipe contains strain-specific modifications for *Clostridium paraputrificum* DSM 661 *

Final pH: 7.0

Final volume: 1000 ml

Ground beef (fat free)	500.00	g
Distilled water	1000.00	ml
NaOH (1 N)	25.00	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 water to a final volume of 1000 ml, and then add:

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
D-Glucose	4.00	g
Cellobiose	1.00	g
Maltose	1.00	g
Starch (soluble)	1.00	g
L-Cysteine HCl, add to make medium anoxic	0.50	g
Agar, if required	15.00	g

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: and either or

Haemin solution	10.00	ml
Vitamin K₁ solution , alternative	10.00	ml
Vitamin K₃ solution	10.00	ml
Haemin	5.00	mg/l

3. To make medium anoxic boil it, cool under 100% N₂ gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under same gas atmosphere 7 ml medium 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 30 min. For agar slants use 15.0 g agar per 1000.0 ml medium.

* Plus haemine (5µg/ml) plus Vit K₁

Haemin solution (from medium 78)

Haemin	50.00	mg
NaOH (1 N)	1.00	ml

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Distilled water	100.00	ml
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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.

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.