

110. CHOPPED MEAT MEDIUM WITH CARBOHYDRATES

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 ₂ HPO ₄	5.0	g
Resazurin	1.0	mg
Glucose	4.0	g
Cellobiose	1.0	g
Maltose	1.0	g
Starch (soluble)	1.0	g

To make medium anoxic boil it, cool under nitrogen atmosphere, add 0.5 g/l cysteine hydrochloride and adjust pH to 7.0. Dispense anoxically 7 ml medium into Hungate 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 g agar per 1000.0 ml medium.

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 (see below)	10.00	ml
Vitamin K ₁ or Vitamin K ₃ solution (see below)	0.20	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 K₁ solution:

Dissolve 0.1 ml of vitamin K₁/K₃ in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K₃ solution:

Dissolve 5 mg/ml of vitamin K₃ in 10 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.