

78c: CHOPPED MEAT MEDIUM FOR FERTIBACTERIUM SP.

Ground beef (fat free)	500.00	g
NaOH (1 N)	25.00	ml
Casein peptone (BD BACTO)	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
Na-fumarate	6.00	g
Na-formate	6.00	g
Clarified rumen fluid , clarified	50.00	ml
Horse serum (inactivated, INVITROGEN)	50.00	ml
Fatty acid mixture	20.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Haemin solution (0.05% w/v)	5.00	ml
Vitamin K₁ solution (0.05% w/v)	2.00	ml
DL-Dithiothreitol (DTT)	0.30	g
Distilled water	700.00	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 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. After autoclaving add fumarate, formate, rumen fluid, horse serum, fatty acids, vitamins, haemin, vitamin K₁ and DTT from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and sterilized by filtration.

Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml

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Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml