DSMZ

78a: CHOPPED MEAT MEDIUM FOR TREPONEMA SP.

This recipe contains strain-specific modifications for *Treponema brennaborense* DSM 12168 *

Final volume: 1055 ml

To the ready medium, add 50 ml/l amino acid solution and 5 ml/l vitamin solution. These solutions must not be prepared under anaerobic conditions.

Main sol. 78	1000.00	ml
Vitamin solution	5.00	ml
Amino acid solution (filter sterilized)	50.00	ml
Glucose	4.00	g/l

* Plus glucose (0.4%) plus bicarbonate plus 0.5 bar hydrogen in the gas space

Amino acid solution (filter sterilized)

L-Histidine	0.60	g
L-Serine	0.50	g
L-Glutamine	0.70	g
Distilled water	50.00	ml

Vitamin solution (from medium 461)

Vitamin B_{12}	50.00	mg
Pantothenic acid	50.00	mg
Riboflavin	50.00	mg
Pyridoxamine hydrochloride	10.00	mg
Biotin	20.00	mg
Folic acid	20.00	mg
Nicotinic acid	25.00	mg
Nicotine amide	25.00	mg
alpha-lipoic acid	50.00	mg
p-Aminobenzoic acid	50.00	mg
Thiamine-HCl x 2 H_2O	50.00	mg
Distilled water	1000.00	ml

Stir for some hours, filter sterilize the solution.

Main sol. 78 (from medium 78)

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_2HPO_4	5.00	g
Resazurin	1.00	mg

2. To make medium anoxic bring it to a boil, cool under 100% N_2 gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under 100% N_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 30 min. For agar slants use 15.0 g agar per 1000.0 ml medium.

L-Cysteine HCl	0.50	g
Agar, for solid medium	15.00	g

3. 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 (optional)	10.00	ml
Vitamin K_1 solution, alternative (optional)	10.00	ml
Vitamin K_3 solution, alternative (optional)	10.00	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 K_3 solution

Vitamin K_3	5.00	mg
Ethanol (95%)	1.00	ml
Water	100.00	ml

Dissolve 5 mg vitamin K_3 in 1 ml 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.

Vitamin K_1 solution (from medium 78)

Vitamin K_1	0.10	ml

Ethanol (95 %)	20.00	ml

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