

## 78: CHOPPED MEAT MEDIUM

This recipe contains strain-specific modifications for *Ruthenibacterium lactatiformans* DSM 105274 \*

Final pH: 7.0

Final volume: 1025 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 <sub>2</sub> HPO <sub>4</sub> | 5.00  | g  |
| Resazurin                       | 1.00  | mg |

2. To make medium anoxic bring it to a boil, cool under 100% N<sub>2</sub> gas atmosphere, add 0.5 g/l L-cysteine hydrochloride and adjust pH to 7.0. Dispense under 100% N<sub>2</sub> 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<sub>1</sub> or Vitamin K<sub>3</sub> is necessary. Add to 1000 ml of medium after autoclaving:

|                                                                |       |    |
|----------------------------------------------------------------|-------|----|
| <b>Haemin solution</b> (optional)                              | 10.00 | ml |
| <b>Vitamin K<sub>1</sub> solution</b> , alternative (optional) | 10.00 | ml |
| <b>Vitamin K<sub>3</sub> solution</b> , alternative (optional) | 10.00 | ml |

\* Plus NBB concentrate; anaerobic (N<sub>2</sub>CO<sub>2</sub> plus 0,5 bar H<sub>2</sub>)

### 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.

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### Vitamin K<sub>1</sub> solution (from medium 78)

|                        |       |    |
|------------------------|-------|----|
| Vitamin K <sub>1</sub> | 0.10  | ml |
| Ethanol (95 %)         | 20.00 | ml |

Dissolve 0.1 ml of vitamin K<sub>1</sub> in 20 ml 95% ethanol and filter sterilize. Store refrigerated in a brown bottle.

### Vitamin K<sub>3</sub> solution

|                        |        |    |
|------------------------|--------|----|
| Vitamin K <sub>3</sub> | 5.00   | mg |
| Ethanol (95%)          | 1.00   | ml |
| Water                  | 100.00 | ml |

Dissolve 5 mg vitamin K<sub>3</sub> in 1 ml 95% ethanol, dilute to 0.05 mg/ml in water and filter sterilize. Store refrigerated in a brown bottle.