

**1637: METHANOMASSILIICOCCUS LUMINYENSIS MEDIUM**

Trypticase peptone (BD BACTO)	16.00	g
Yeast extract (OXOID)	7.00	g
NaCl	5.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
NaHCO <sub>3</sub>	0.50	g
<b>Haemin solution</b>	5.00	ml
<b>Vitamin K<sub>1</sub> solution</b>	5.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Methanol (15% v/v)	10.00	ml
DL-Dithiothreitol	1.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients except for methanol and dithiothreitol. Adjust pH to 7.5 with 1 N NaOH and sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Distribute medium under 100% N<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add methanol from a sterile anoxic stock solution (15% v/v) autoclaved under 100% N<sub>2</sub> gas atmosphere and dithiothreitol from an anoxic stock solution sterilized by filtration. Before use check the pH of the complete medium and adjust it to 7.6 - 7.8, if necessary.

2. After inoculation, add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 0.5 bar overpressure. After growth becomes visible overpressure of 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture can be increased to 1 bar.

3. Note: Use 10% (v/v) as inoculum.

**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<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.