

## 1132. LC 2

MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.20	g
KNO <sub>3</sub>	0.05	g
SL-10 (see below)	1.00	ml
H <sub>2</sub> O	1000.00	ml

Dispense 5 ml aliquots into Hungate tubes. After autoclaving add the following components per liter:

Phosphate buffer (pH 6.8, see below)	2.0	ml
2 M HEPES buffer (pH 7.0)	1.0	ml
Fe-NH <sub>4</sub> EDTA (4mg/ml)	1.0	ml
Methanol	0.3	ml

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### *Trace element solution SL-10:*

HCl (25%; 7.7 M)	10.0	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.5	g
ZnCl <sub>2</sub>	70.0	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.0	mg
H <sub>3</sub> BO <sub>3</sub>	6.0	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.0	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.0	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.0	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.0	mg
Distilled water	990.0	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

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### *Phosphate buffer:*

Na <sub>2</sub> HPO <sub>4</sub>	3.6	g
KH <sub>2</sub> PO <sub>4</sub>	1.4	g
H <sub>2</sub> O	100.0	ml

Adjust to pH 6.8.

For DSM 22980 after autoclaving add 1 ml of vitamine solution 141 (see Medium141) to 1 litre of the sterile medium.

Incubate in sealed containers. The strain held in the DSMZ has been cultivated on methanol, but the original publication indicates that the strain will also grow on methane.