

## 863. METHANOTHERMOBACTER/METHANOBACTERIUM TRANSDUCTION MEDIUM

Basal salts solution	100.000	ml
Trace elements solution	10.000	ml
Sodium carbonate solution	24.000	ml
Cysteine solution	5.000	ml
Na <sub>2</sub> S solution	5.000	ml
Degassed distilled H <sub>2</sub> O	856.000	ml

Anaerobically dispense liquid minimal medium in 10-ml portions into 100-ml serum vials under an atmosphere of N<sub>2</sub>/CO<sub>2</sub> (80:20). Seal vials with butyl rubber stoppers secured with aluminium crimp seals. Sterilize by autoclaving for 15 minutes at 121°C. Prior to inoculation, pressurize bottles to 2 x 10<sup>2</sup> kPa with H<sub>2</sub>/CO<sub>2</sub>. For solidified plating medium, add the following components per liter of liquid minimal medium:

Agar	15.000	g
Titanium citrate solution	16.000	ml

Add titanium(III) citrate solution to warm (60°C) agar medium after autoclaving. For preparation of solidified agar medium.

### *Basal salts solution:*

KH <sub>2</sub> PO <sub>4</sub>	68.000	g
NH <sub>4</sub> Cl	21.200	g
Resazurin	10.000	mg
Distilled or deionized H <sub>2</sub> O	1000.000	ml

### *Trace elements solution:*

Nitrilotriacetic acid (Aldrich N8407)	9.550	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.060	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.980	g
NaWO <sub>4</sub> x 6 H <sub>2</sub> O	0.264	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.118	g
Na <sub>2</sub> Se <sub>2</sub> O <sub>3</sub>	0.100	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.024	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.023	g
Distilled or deionized H <sub>2</sub> O	1000.000	ml

Adjust pH of nitrilotriacetic acid in solution to 7.0 with KOH. Add minerals and adjust the final pH to 7.0.

*Continued next page*

*Na<sub>2</sub>CO<sub>3</sub> solution:*

Dissolve 106 g Na<sub>2</sub>CO<sub>3</sub> in 1 liter of degassed H<sub>2</sub>O. Autoclave in sealed serum bottles under CO<sub>2</sub>.

*Cysteine and Na<sub>2</sub>S solutions:*

To 50 ml of boiled and cooled distilled or deionized H<sub>2</sub>O add 5 g each of L-cysteine hydrochloride and Na<sub>2</sub>S x 9 H<sub>2</sub>O. Adjust pH to 10 with 3N NaOH and store under N<sub>2</sub> in a serum bottle sealed with a butyl rubber stopper.

*Titanium (III) citrate solution (Zehnder and Wuhrmann 1976):*

Titanium(III) chloride, 15% solution (Merck 808307)	5.000	ml
Sodium citrate, 0.2M	50.000	ml

Neutralize pH with a saturated sodium carbonate solution and store in a serum bottle under N<sub>2</sub>.