**M9 minimal medium**

**M9 10x:**

Na2HPO4 (A44) 239 mM -> 33.90 g 27.12 g 54.24g

KH2PO4 (A30) 220 mM -> 15 g 12 g 24g

NaCl (A14) 85.6 mM -> 2.5 g 2 g 4g

NH4Cl (A19) 187 mM -> 5 g 4 g 8g

dH2O to 500 ml 400 ml 800 ml

* pH= 7,4
* Autoclavar

**For 1x M9 minimal medium for *P. putida* Volumen = 50 ml**

M9 10x = 5 ml [1x]

MgSO4 1M = 100 μl [2mM]

goodies 1000x = 50 μl [1x]

carbon source 20% = 500 μl [0.2%]

dH2O to 50 ml

**For 1x M9 minimal medium (50 ml) for *E. coli***

5 ml M9 10x

100 μl MgSO4 1M

50 μl goodies 1000x

50 μl Thiamine 0.2 %

500 μl carbon source 20%

dH2O to 50 ml

(if necessary add 5 μl of B12, 1 mg/ml) **For plates add Agar to 1.5%**

**For 1x M9 minimal medium (20 ml) for *E. coli* DH5a**

M9 10x -> 2 ml

MgSO4 1M -> 40 μl

Goodies 1000x -> 20 μl

Thiamine 0.4 % -> 10 μl

Casamino acids 20 % -> 20 μl

Carbon source 20% -> 200 μl

dH2O to 20 ml

(if necessary add 5 μl of B12, 1 mg/ml) **For plates add Agar to 1.5%**

**M8 minimal medium**

**M8 10X**

Na2HPO4 (A44) 239 mM -> 33.90 g 27.12 g 54.24g

KH2PO4 (A30) 220 mM -> 15 g 12 g 24g

NaCl (A14) 85.6 mM -> 2.5 g 2 g 4g

dH2O to 500 ml 400 ml 800 ml

* Autoclavar

|  |  |  |
| --- | --- | --- |
| **MOPS Minimal Media (M3)** | | |
| **A. M3 10x stock ( for 1 L)** | | |
| **reagent** | | **Quantity** |
| MOPS ( Sigma-Aldrich, Cat# M1254) | | 83.72 g |
| Tricine (Sigma-Aldrich, Cat# T0377) | | 7.17 g |
| sterile ddH2O to | | 300 ml |
| adjust pH to 7.4 with KOH 5M. add in order | | |
| FeSO4.7H2O (0.01 M o 2.78 g/l) (fresh) | | 10 ml |
| NH4Cl (1.9M) | | 50 ml |
| K2SO4 (0.276M) | | 10 ml |
| CaCl2 (1000x, 44 g/l, 0.396 M) | | 10 ml |
| MgCl2 2.5M | | 2.1 ml |
| NaCl (5M) | | 100 ml |
| Micronutient stock (trace elements 1000X\*) | | 5 ml |
| If necessary adjust the pH to 7.2 with NaOH 5M | | |
| sterile ddH2O to | | 1000 ml |
| **Filter sterilize with 0.22 micron** | | |
|  | **B. M3 ( for 400 mL)** | | | |
|  |  | | | |
|  | **reagent** | | | **Quantity** |
|  | M3 10x stock | | | 40 ml |
|  | Glucose 20% | | | 4 ml |
|  | Vitamin solution 1000X \*\* | | | 400 μl |
|  | antibiotic Kn 50 mg/ml | | | 400 μl |
|  | Agar pure 3% (for M3 agar plates) | | | (200 ml) |
|  | sterile ddH2O to | | | To 400 ml |
| **B. M3 ( for 400 mL) (for E. coli HD10B)** | |
|  | |
| **reagent** | **Quantity** |
| M3 10x stock | 40 ml |
| Glucose 20% | 4 ml |
| Vitamin solution 1000X \*\* | 400 μl |
| Casamino acids 10% (if necessay, p.e. with DH10B) | (800 μl) |
| antibiotic (pe. Kn 50 mg/ml), if necessary | (400 μl) |
| Agar pure 4% (for M3 agar plates) | (200 ml) |
| sterile ddH2O to | To 400 ml |

|  |  |
| --- | --- |
| **\* Trace elements solution:** | |
| **component** | **1000X (g/l)** |
| Acido nitrilotriacético (NTA) | 1,5 |
| MgSO4. 7H2O | 3 |
| MnSO4·H2O | 0,5 |
| NaCl | 1 |
| FeSO4.7H2O | 0,1 |
| CoSO4 7H2O | 0,18 |
| Cl2Ca 2H2O | 0,1 |
| ZnSO4 . 7H2O | 0,18 |
| CuSO4.5H2O | 0,01 |
| AlK(SO4)2 . 12H2O | 0,02 |
| H3BO3 | 0,01 |
| Na2MoO4·2H2O | 0,01 |
| NiCl2 | 0,025 |
| Na2SeO4 | 0,3 |

|  |  |
| --- | --- |
| **\*\* Vitamin solution:** |  |
| **component** | **1000X** |
| Vitamin B12 (cobalamin) | 25mg |
| Pantothenic acid(calcium salt) | 25mg |
| Riboflavin | 25mg |
| Pyridoxine Hydrochloride | 5mg |
| Biotin(vit H) | 10mg |
| Folic acid | 10mg |
| Nicotinic acid | 12,5mg (25mg) |
| P-aminobenzoic acid | 25mg |
| Thiamine hydrochloride | 25mg |
| sterile ddH2O | 500 ml |

**GOODIES 1000X** (quedan rojizos y con el tiempo se vuelven amarillentos)

**100 mL** **1L**

**FeSO4.7H2O [10mM] (A29)** 0.278g 2,78g

**MnCl2.4H2O (A4)** 0.198g 1,98g

**CoCl2.6H2O [10mM] (A51)** 0.238g 2,38g

**CaCl2.2H2O (A11)** 0.147g 1,47g

**CuSO4.5H2O [1mM] (A38)** 0.025g 0,25g

Z**nSO4.7H2O (A36)** 0.029g 0,29g

**HCl 1N fumante hasta** 100 mL 1000ml

· Disolver en HCl 1N. No hace falta esterilizar.

**m-toluic 0.5 M**

V final = 100 mL

pesar 6.81 gr, añadir 20-21 perlas NaOH, esperar a que se disuelva todo. Ajustar pH = 8