**Table 11.** Modified NMS ("P") medium for methanotrophicstrains

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Solution 1** | **NMS -P0%** | **NMS-P0.1%** | **NMS-P0.75%** | **NMS-P3%** | **Comments/notes** |
| g/L | g/L | g/L | g/L |
| KNO3 | 1 g | 1 g | 1 g | 1 g | Autoclave at 121oC for 30 minutes.  |
| MgSO4 x 7H2O | 0.2 g | 0.2 g | 0.2 g | 0.2 g |
| CaCl2 x 2H2O | 0.02 g | 0.02 g | 0.02 g | 0.02 g |
| NaCl    | - | 1g | 7.5 g | 30 g |
| Trace solution  | 1 ml | 1 ml | 1 ml | 1 ml |
| Water (dH2O) | up to 1L | up to 1L | up to 1L | up to 1L |
| **Solid medium**  |
|  Solution 1  | 150 ml | Autoclave at 121oC for 30 minutes.  |
|  Agar | 1.75 g |
| **Phosphate solution** (pH 6.8) | 20 ml/L | Phosphate and carbonate solutions should be prepared separately, and mix with the medium just before inoculation. NOTE: never add phosphate and/or carbonate solution to hot media. Media should be cold or warm (for making agar plates). |
| **Carbonate solution** (1M, pH 8.6-9.2) | 40 ml/L |
| **Trace solution** |
|  **(1000x)** | g/L | Autoclave at 121oC for 30 minutes. After autoclaving the solution becomes pink.NOTE: trace solution should not have a precipitate. If a precipitation will occur make a new stock. |
| Na2EDTA | 5 |
| FeSO4 x 7 H2O | 2 |
| ZnSO4 x7 H2O | 0.3 |
| MnCl2 x 4 H2O     | 0.03 |
| CoCl2 x 6 H2O       | 0.2 |
| CuSO4 x 5 H2O   | 1.2 |
| Na2O4W x 2H2O | 0.3 |
| NiCl2 x 6 H2O       | 0.05 |
| Na2MoO4 x 2 H2O   | 0.05 |
| H3BO3 | 0.03 |
| Water (dH2O) | up to 1 L |
| **Phosphate solution** **(pH 6.8)** | g/L | pH of the solution should be 6.8-7. Autoclave at 121oC for 20 minutes. |
| KH2PO4 | 5.445.68 |
| Na2HPO4 |
| **Carbonate solution (1M, pH 8.8-9.2)** | g/L | pH of the solution should be 8.6-9.0. NOTE: Sterilize by filtration only. |
| NaHCO3 | 75.6 |
| Na2CO3 | 10.5 |

 REVISED 11/24/2021

**General** “P”**-medium Preparation Guidelines**

Preparing liquid culture.

1. take 150 ml of P0.75% or P3% medium;
2. Add 3 ml of phosphate solution;
3. Add 6 ml of carbonate solution;
4. Mix and transfer 50 ml of the solution into 125 ml bottles or vials;
5. Add 1-5 ml of inoculum per bottle or vial;
6. Close tightly and add 50 ml of methane;
7. Incubate on shaker at 30oC with shaking (200 rpm) for 16-48h.

Preparing agar plates.

1. Take 150 ml of PA0.75% or PA3% medium;
2. Melt in a microwave (2 -2.5 min);
3. Cool till it is warm;
4. Add 3 ml of phosphate solution;
5. Add 6 ml of carbonate solution;
6. If needed, add antibiotic;
7. Pour 6 plates.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Medium  |  Phosphate solution | Carbonate solution | Rifamycin(if needed)Stock solution 100 mg/ml | Kanamycin(if needed)Stock solution 100 mg/ml | Methanol(if needed) | LB or NB media |
| **High pH (8.6-9.2) P0.75% or P3% medium and PA0.75% or PA3%**  |
| 100 ml | 2 ml | 4 ml | 0.05 ml | 0.1 ml | 0.2 ml | - |
| 150 ml | 3 ml | 6 ml | 0.075 ml | 0.15 ml | 0.3 ml | - |
| 200 ml | 4 ml | 8 ml | 0.1 ml | 0.2 ml | 0.4 ml | - |
| 300 ml | 6 ml | 12 ml | 0.15 ml | 0.3 ml | 0.6 ml | - |
| 1000 ml (1L) | 20 ml | 40 ml | 0.5 ml | 1 ml | 2 ml | - |
| **Low pH (6.8) P0 % or PA0% medium** |
| 100 ml | 5 ml | - | 0.05 ml | 0.1 ml | 0.2 ml | - |
| 150 ml | 7.5 ml | - | 0.075 ml | 0.15 ml | 0.3 ml | - |
| 200 ml | 10 ml | - | 0.1 ml | 0.2 ml | 0.4 ml | - |
| 300 ml | 15 ml | - | 0.15 ml | 0.3 ml | 0.6 ml | - |
| **Mating or Control plates (use P 0.1% medium), pH 7.2-7.6** |
| 100 ml | 5 ml | 0.5 ml | - | - | - | 10 ml |
| 150 ml | 7.5 ml | 0.75 ml | - | - | - | 15 ml |
| 200 ml | 10 ml | 1 ml | - | - | - | 20 ml |
| 300 ml | 15 ml | 1.5 ml | - | - | - | 30 ml |

# MEDIA RECIPES[[1]](#footnote-1)

## L-Agar 150ml 500ml

### LB Broth 3.75 g 12.5 g

### Bacto Agar 2.7 g 9 g

### ddH2O To the line

## L-Broth 150ml 500ml

### Same as above minus Bacto Agar

## Nutrient Agar 150ml 500ml

### Nutrient Agar 3.68 g NA

ddH2O To the line

## Nutrient Broth 150ml 500ml

### Nutrient Broth 3.68 NA

ddH2O To the line

## TGY Agar 150ml 500ml

### Tryptone-Peptone 0.75 g 2.5 g

Yeast Extract 0.45 g 1.5 g

Glucose (Dextrose/D-Glucose) 0.15 g 0.5 g

Bacto Agar 2.25 g 7.5 g

ddH2O To the line

## TGY Broth 150ml 500ml

### Same as above minus Bacto Agar

## NMS Agar (NMSA) 150ml 500ml

### Bacto Agar 2.7g 9g

### NMS Liquid To the line

### **TBE (1X) 5X) (10X)**

### Tris Base 10.8 g 54 g 108 g

Na2EDTA 0.93 g 4.65 g 9.3 g

Boric Acid 5.5 g 27.5 g 55 g

## “P” Agar (PA) 150ml 500ml

### Bacto Agar 2.7g 9g

### NMS-Px% Liquid To the line

## Hypho Agar 150ml 500ml

### Bacto Agar 2.7g 9g

### Hypho Liquid To the line

**Hypho medium:**

V. Vishniac and M. Santer. *Thiobacilli. Bacteriol. Rev.* **21** (1957), p. 195.

K2HPO4 2.5g/L

NaH2PO4 2.25 g/L

(NH4)2SO4 0.5 g/L

MgSO4x7H2O 0.2g/L

*Visniac Trace Elements* 1 ml

Succinate (or methanol) 0.5g/L (or 1ml/L for methanol)

For agar medium add:

Bacto Agar 15g/L

***Visniac Trace Elements :***

Na2-EDTA\*2H2O 5g

ZnSO4x7H2O 2.2g

CaCl2x2H2O 0.733g

MnCl2x4H2O 0.506g

FeSO4x7H2O 0.499g

(NH4)6Mo7O24x4H2O 0.11g

CuSO4x5H2O 0.157g

CoCl2x6H2O 0.161g

1. REVISED 11/24/2021 [↑](#footnote-ref-1)