

## 1668. MDM (modified dehority medium)

Yeast Extract	0.50	g
Tryptone	0.50	g
Stock A	250	ml
Stock B	2.5	ml
Distilled water	250	ml

To make medium anoxic boil it, cool under  $N_2CO_2$  gas atmosphere, add 1 g/l L-cysteine hydrochloride and adjust pH to 6.8. Finally gas over with  $N_2$ . Dispense under  $N_2CO_2$  into suitable vessels for anaerobic growth (serum bottles or Hungate tubes sealed with rubber stoppers) and sterilize by autoclaving.

### For **DSM 104696, DSM 104698, DSM 104699**

For 10 ml in hungate tube, add:

Vitamin solution	5-10	$\mu$ l
Rumen fluid	500	$\mu$ l
Cellobiose (10%)	200	$\mu$ l

### For **DSM 104697**

For 10 ml in hungate tube, add:

Vitamin solution	5-10	$\mu$ l
Rumen fluid	500	$\mu$ l
Cellobiose (10%)	200	$\mu$ l
CM-Mix	500	$\mu$ l
Vitamin K1 solution	5-10	$\mu$ l

### **Stock A:** Salt solution 2x (50 ml stock per 100 ml medium)

$K_2HPO_4$	6804	mg
$NaHCO_3$	6721	mg
NaCl	4675	mg
$NH_4Cl$	1605	mg
$CaCl_2$	89	mg
$MgCl_2$	95	mg
$FeSO_4 \times 7 H_2O$	28	mg
Distilled water	1000	ml

**Stock B:** Salt solution 200x (0,5 ml stock per 100 ml medium)

MgCl <sub>2</sub> x 2 H <sub>2</sub> O	971	mg
ZnCl <sub>2</sub>	273	mg
CoCl <sub>2</sub>	104	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	48	mg
Na <sub>2</sub> SeO <sub>3</sub>	35	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	237	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	66	mg
Distilled water	1000.00	ml

**Vitamin solution:**

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B12	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

Filter sterilize and make anoxic with N<sub>2</sub>.

**Cellulose (10%)**

Mix 10 g cellulose with 100 ml distilled water and filter sterilize, make anoxic with N<sub>2</sub>.

**CM-Mix (only for DSM 104697)**

D-Glucose	4.0	g
Cellulose	1.0	g
Maltose	1.0	g
Starch (soluble)	1.0	g
Distilled water	1000.00	ml

Make anoxic with N<sub>2</sub>.

**Vitamin K<sub>1</sub> solution (only for DSM 104697)**

Dissolve 0.1 ml of vitamin K<sub>1</sub> in 20 ml 95% ethanol and filter sterilize. Make anoxic with N<sub>2</sub>. Store refrigerated in a brown bottle.