

ATCC Medium: 2752 *Elusimicrobium minutum* medium

Base Medium

KCl.....	0.335 g
MgCl ₂ x7H ₂ O.....	2.75 g
MgSO ₄ x7H ₂ O.....	3.45 g
NH ₄ Cl.....	0.25 g
CaCl ₂ x2H ₂ O.....	0.14 g
K ₂ HPO ₄	0.14 g
NaCl.....	1.0 g
Trace Elements Solution SL-6 (See Below).....	10.0 ml
Wolfe's Vitamin Solution (See Below).....	10.0 ml
Fe(NH ₄) ₂ (SO ₄) ₂ x7H ₂ O.....	2.0 mg
NaHCO ₃	5.0 g
Glucose.....	0.18 g
Yeast extract.....	0.2 g
Casamino acids.....	0.2 g
Resazurin.....	1.0 mg
L-Cysteine HCl x H ₂ O.....	0.5 g
DTT Solution (See Below).....	10ml
DI Water.....	970 ml

Adjust for final pH 7 – 7.5. Prepare anaerobically under 80% N₂ - 20% CO₂.
Autoclave at 121°C.

DTT Solution

Dithiothreitol (DTT).....	0.368 g
DI Water.....	10.0 ml

Weigh the DTT and place the crystals into a sterile test tube. Seal and exchange the head space for 80 % N₂ – 20% CO₂. Wait for the crystals to dissolve and then filter sterilize directly into the sterilized medium. Under anaerobic conditions distribute the medium into sterile Balch tubes and seal.

To store:

Stock 100 mM Dithiothreitol solution

Dithiothreitol 0.154 g dissolved in 10 ml of distilled water then filter sterilized into a Balsh tube and sealed This solution can be used to reduce the medium after storage. Use 0.1 ml for each 10 ml of medium.

Trace Elements Solution SL-6

(ATCC MD-TMS can be substituted for Trace Elements SL-6)

ZnSO ₄ x 7H ₂ O.....	0.10 g
MnCl ₂ x 4H ₂ O.....	0.03 g
H ₃ BO ₃	0.3 g
CoCl ₂ x 6H ₂ O.....	0.2 g

CuCl ₂ x 2H ₂ O.....	0.01 g
NiCl ₂ x6H ₂ O.....	0.02 g
Na ₂ MoO ₄ xH ₂ O.....	0.03 g
DI Water.....	1000 ml

Adjust final pH of Trace Elements Solution SL-6 to 3.4.

Wolfe's Vitamin Solution

(Available from ATCC as a sterile ready-to-use liquid Vitamin Supplement, catalog no. MD-VS).

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine hydrochloride.....	10.0 mg
Thiamine. HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
Calcium D-(+)-pantothenate.....	5.0 mg
Vitamin B12.....	0.01 mg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg