

ATCC Medium 2654: Clostridium OhILA's freshwater Medium

K ₂ HPO ₄	0.225 g
KH ₂ PO ₄	0.255 g
NaCl	0.460 g
MgCl ₂	0.255 g
Yeast extract	5.0 g
NaHCO ₃	4.20 g
Sodium sulfide	0.10 g
ATCC vitamins	10.0 mL
ATCC trace mineral solution	10.0 mL
Distilled water	980.0 mL

Add the appropriate substrates (see below) prior to inoculating the medium.

*Distribute medium into Balch tube, stopper and seal with aluminum crimp and gas with 100 % N₂. Autoclave at 121 °C for 15 minutes. **Final pH should be 6.8.***

Add substrate separately.

Substrates:

Na-arsinate, 1M (1.85 g in 10 ml distilled water)

Na-lactate, 2M (2.24 g in 10 ml distilled water)

Fructose, 2M (3.6 g in 10 ml distilled water)

Thiosulfate, 1M (1.58 g in 10 ml)

Make substrates, filter sterilize and put each into a separate sterile Balch tube. Stopper, seal, and replace the head space with 100% nitrogen.

ATCC® BAA-1360™ is a versatile strict anaerobe which is able to ferment lactate (20mM) and fructose (20mM) and respire arsenate (10mM) and thiosulfate (10mM). BAA-1360™ was isolated using a freshwater medium containing sodium arsenate and sodium lactate.

ATCC Trace Mineral Supplement catalog no. MD-TMS

Available from ATCC as a sterile ready-to-use liquid.

ZnSO ₄ ·7H ₂ O	0.10 g
MnCl ₂ ·4H ₂ O	0.03 g
H ₃ BO ₃	0.3 g
CoCl ₂ ·6H ₂ O	0.2 g
CuCl ₂ ·2H ₂ O	0.01 g
NiCl ₂ ·6H ₂ O	0.02 g
Na ₂ MoO ₄ ·H ₂ O	0.03 g
Distilled water	1.0 L

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

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

Biotin	2.0 mg
Folic acid	2.0 mg
Pyridoxine HCl	10.0 mg
Thiamine HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
Calcium D-(+)-pantothenate	5.0 mg
Cyanocobalamine	100.0 mcg
<i>p</i> -Aminobenzoic acid	5.0 mg
Thioctic acid	5.0 mg
Distilled water	1.0 L

CONDITIONS:

Temperature: 18-23°C

Atmosphere: Anaerobic gas mixture,

100% N₂ or 80% N₂-20%CO₂