

**ATCC medium: 1004 Sweet E broth for anaerobes**

Rumen fluid.....	30.0 ml
L-Arabinose.....	0.1 g
Cellobiose.....	0.1 g
Fructose.....	0.1 g
Glucose.....	0.1 g
Maltose.....	0.1 g
Starch.....	0.1 g
Pyruvic acid.....	0.01 ml
Gelatin.....	0.3 g
Agar.....	0.075 g
Peptone.....	0.05 g
Yeast extract.....	0.05 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.05 g
Resazurin Solution (see below).....	0.4 ml
Salts Solution (see below).....	50.0 ml
Distilled water.....	20.0 ml
L-Cysteine . HCl.....	0.05 g

Adjust final pH to 6.5.

Bring the medium to a boil while bubbling with oxygen-free gas (97% N<sub>2</sub>, 3% H<sub>2</sub>). Check and adjust the pH of the medium periodically while it boils. After it becomes colorless, tube it under the same gas phase and cap with butyl rubber stoppers. Place the tubes in a press and autoclave at 121C for 15 minutes.

*Resazurin Solution:*

Resazurin.....	25.0 mg
Distilled water.....	100.0 ml

*Salts Solution:*

CaCl <sub>2</sub> (anhydrous) .....	0.2 g
MgSO <sub>4</sub> .....	0.2 g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0 g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0 g
NaHCO <sub>3</sub> .....	10.0 g
NaCl.....	2.0 g

Dissolve CaCl<sub>2</sub> and MgSO<sub>4</sub> in 300 ml of distilled water. Add 500 ml water and add the remaining salts while swirling slowly. Add 200 ml of distilled water, mix, and store at 4C.