

### **ATCC Medium: 1494 Modified NOS Medium**

Heart Infusion Broth (BD 238400).....	1.25 g
Trypticase Peptone (BD 211921).....	1.0 g
Yeast Extract (BD 211928).....	0.25 g
Sodium thioglycollate.....	0.05 g
L-Cysteine x HCl.....	0.1 g
L-Asparagine.....	0.025 g
D-Glucose.....	0.2 g
Resazurin (0.1% w/v in water).....	0.1 ml
DI Water to.....	94.0 ml

Combine the above ingredients and bring to a boil in a round-bottom flask while flushing with O<sub>2</sub>-free gas (10% H<sub>2</sub>, 10% CO<sub>2</sub>, 80% N<sub>2</sub>). Stopper anaerobically with butyl rubber, wire or seal closed, and autoclave at 121°C. After autoclaving, allow to cool, open without admitting oxygen and while flushing with gas, add the following filter-sterilized ingredients:

NaHCO <sub>3</sub> (7.5% w/v in water).....	2.67 ml
TPP/VFA Solution (see below).....	2.0 ml
Rabbit serum.....	2.0 ml

#### **TPP-VFA Solution**

Thiamine pyrophosphate (0.2% w/v in water).....	1.5 ml
VFA Solution (see below).....	1.0 ml
DI Water.....	8.4 ml

Mix together and filter-sterilize. Store at -20C.

#### **VFA Solution**

Isobutyric acid.....	0.5 ml
2-Methylbutyric acid.....	0.5 ml
Isovaleric acid.....	0.5 ml
Valeric acid.....	0.5 ml
0.1 N NaOH to.....	100.0 ml

Add volatile fatty acids to about 70-80 ml of NaOH and mix. Add NaOH to bring final volume to 100 ml. If not used the same day, filter-sterilize and store at 4C.