

**ATCC medium: 1367 Modified OTI medium**

Polypeptone Peptone (BD 211910).....	5.0 g
Heart infusion broth.....	5.0 g
Yeast Extract (BD 212750).....	5.0 g
Pectin (Sigma P9135).....	0.8 g
Glucose.....	0.8 g
Starch.....	0.8 g
Sucrose.....	0.8 g
Maltose.....	0.8 g
Sodium pyruvate.....	0.8 g
Ribose.....	0.8 g
K <sub>2</sub> HPO <sub>4</sub> .....	2.0 g
NaCl.....	5.0 g
MgSO <sub>4</sub> .....	0.1 g
L-Cysteine . HCl.....	0.68 g
Clarified rumen fluid.....	500.0 ml
Agar.....	1.6 g
Distilled water.....	500.0 ml

Adjust pH to 7.0. Prepare medium under anaerobic conditions and tube in butyl rubber-stoppered tubes under oxygen-free nitrogen. Add inactivated sterile rabbit serum to give final concentration in each tube of 5%. Add filter-sterilized thiamine pyrophosphate to each tube to give a final concentration of 0.75 mg/100 ml. Subculture with a Pasteur pipette (20-30 drops). Gas phase for culture system should be oxygen-free nitrogen or a mixture of 90% N<sub>2</sub> and 10% CO<sub>2</sub>.