

**ATCC medium: 983 E medium for anaerobes with 0.2% rutin**

ATCC Medium 982 (see below) with 1% phloroglucinol replaced by 0.2% rutin.

**ATCC Medium 982:**

Rumen Fluid (see below).....30.0 ml  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.....0.5 g  
Resazurin Solution (see below).....0.4 ml  
Salt Solution (see below).....50.0 ml  
L-Cysteine . HCl.....0.05 g  
Sterile 1% phloroglucinol solution.....30.0 ml

Mix all the ingredients in an Erlenmeyer flask. Flask should have a small head space to minimize air volume that must be purged during cooling. Fit a removable chimney to the boiling flask to prevent medium from boiling over. Boil (10-20 min) until medium changes from pink to yellow. Replace chimney with stopper fitted with glass tubing which bubbles O<sub>2</sub>-free gas, composed of N<sub>2</sub> and H<sub>2</sub> through the medium to maintain in reduced state (97% N<sub>2</sub>, 3% H<sub>2</sub> is employed at the ATCC) and place in an ice bath medium into tubes under O<sub>2</sub>-free N<sub>2</sub> and H<sub>2</sub>. Stopper with butyl rubber stoppers. Place rack of tubes in press so that stoppers do not dislodge, and autoclave at 121C for 12-15 minutes on fast exhaust.

*Rumen Fluid:*

Filter rumen contents, obtained from a cow fed an alfalfa-hay concentrated ration, through two layers of cheesecloth to remove large particles. Store bottles in the freezer. Much of the particulate matter settles. Use only the supernatant.

*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.

*Phloroglucinol:*

Filter-sterilize freshly prepared phloroglucinol solution. Wrap glassware in aluminum foil to exclude light.