ATCC medium: 982 E medium for anaerobes with 0.3% phloroglucinol

Rumen Fluid (see below).....30.0 ml
$(NH_4)_2SO_4$.....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 headspace 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_2-free gas, composed of N_2 and H_2 through the medium to maintain in reduced state (97% N_2, 3% H_2 is employed at the ATCC) and place in an ice bath medium into tubes under O_2-free N_2 and H_2. 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:
$CaCl_2$ (anhydrous) .....0.2 g
$MgSO_4$ .....0.2 g
K_2HPO_4 .....1.0 g
KH_2PO_4 .....1.0 g
$NaHCO_3$ .....10.0 g
NaCl.....2.0 g

Dissolve $CaCl_2$ and $MgSO_4$ 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 Solution:
Filter-sterilize freshly prepared phloroglucinol solution. Wrap glassware in aluminum foil to exclude light.