

ATCC Medium: 1365 E Medium for Anaerobes With 0.1% Cellobiose

Rumin fluid (see below)	30.0	ml
Glucose	0.05	g
Maltose	0.05	g
Soluble Starch	0.05	g
Peptone	0.05	g
Yeast Extract	0.05	g
Cellobiose	0.1	g
(NH ₄) ₂ SO ₄	0.04	g
Resazurin Soln. (see below)	1.0	mg
Salts Solution (see below)	50.0	ml
L-Cystein x H ₂ O	0.05	g
DI Water	20.0	ml

Rumin Fluid:

Filter rumen contents, obtained from a cow fed on an alfalfa-hay concentrate ration, through two layers of cheesecloth to remove large particles. Store under CO₂ in quart milk bottles in the refrigerator. Much of the particulate matter settles. Use only the supernatant.

Resazurin Solution:

Dissolve one resazurin tablet in 44 ml of distilled water.

Salts Solution:

CaCl ₂ (anhydrous)	0.2	g
MgSO ₄	0.2	g
K ₂ HPO ₄	1.0	g
KH ₂ PO ₄	1.0	g
NaHCO ₃	10.0	g
NaCl	2.0	g

Mix CaCl₂ and MgSO₄ in 300 ml of distilled water until dissolved. Add 500 ml water and add the remaining salts while swirling slowly. Add 200 ml of distilled water, mix and store at 4 C. Mix all of the ingredients in an Erlenmyer flask. Flask should have a small heat space to minimize air volume that must be purged during cooling. Fit a removable chimney to the boiling flask to prevent media from boiling over. Boil (10-20 minutes) until medium changes from pink to yellowish. Cool in ice water bath under O₂-Free CO₂. The flow of CO₂ should cause gentle bubbling (sufficient to exclude air). Remove from ice bath and add 0.05 g L-cysteine x H₂O. Adjust pH to 7.0 with 8N NaOH or 5N HCl. Stopper with #1 butyl rubber stoppers (or black rubber stoppers). The nitrogen prevents a pH change

during storage. Place the rack of tubes in a press and sterilize for 12-15 minutes at 121 C.