

ATCC Medium: 602 E Medium for Anaerobes

Base Medium

Rumen fluid (see below).....	300	ml
Glucose.....	0.5	g
Maltose.....	0.5	g
Soluble Starch.....	0.5	g
Peptone.....	0.5	g
Yeast Extract.....	0.5	g
(NH ₄) ₂ SO ₄	0.4	g
Resazurin Solution (see below).....	4.0	ml
Salts Solution (see below).....	500	ml
L-Cysteine x H ₂ O.....	0.5	g
DI Water.....	200	ml

Mix all of the ingredients in an anaerobe jar. Bring jar to a gentle boil. Cool under 80:20 gas for approximately 10-20 minutes until medium changes from pink to yellowish. The flow of gas should cause gentle bubbling (sufficient to exclude air). Add 0.5 g L-cysteine x H₂O. Adjust pH to 7.0 if required with 6N NaOH or 5N HCl. Dispense anaerobically and stopper with butyl rubber stoppers. Autoclave at 121°C for 15-20 minutes.

Rumen 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 11mg resazurin 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