

ATCC Medium: 158 RGCA Medium

Complete Medium

Solution 1 (see below).....	150 ml
Solution 2 (see below).....	150 ml
Solution 3 (see below).....	1 ml
Solution 4 (see below).....	220 ml
Rumen Fluid (see below)	400 ml
Agar (if needed).....	15 g

Autoclave at 121°C. Allow to cool while stirring under CO₂ gas. Aseptically add:

Solution 5 (see below).....	17 ml
Solution 6 (see below).....	66 ml

After continued gassing for 15 minutes, adjust pH to 6.6. Dispense aseptically into sterile gassed out tubes.

Solution 1

K ₂ HPO ₄	1.5 g
DI Water.....	500 ml

Solution 2

KH ₂ PO ₄	1.5 g
(NH ₄) ₂ SO ₄	3.0 g
NaCl.....	3.0 g
MgSO ₄	0.3 g
CaCl ₂	0.3 g
DI Water.....	500 ml

Solution 3

Resazurin.....	0.05 g
DI Water.....	50 ml

Solution 4

Glucose.....	3.0 g
Cellobiose.....	3.0 g
DI Water.....	325 ml

Rumen Fluid

Filter rumen contents through two layers of cheesecloth to remove large particles. Use only the supernatant. Centrifuge the rumen fluid at 9500rpm for 15 minutes. Additionally, filter the supernatant through a 0.22-micron filter and add the liquid contents to the base medium.

Solution 5

Cysteine HCl.....0.75 g
DI Water.....25 ml

Boil briefly under CO₂ gas. Seal and autoclave at 121°C.

Solution 6

Na₂CO₃.....6.0 g
DI Water.....100 ml

Boil briefly under CO₂ gas. Seal and autoclave at 121°C.

NOTE

Final media color can be varied from light tan to dark brown/black. It is normal to see color differences with preparations of this media and it appears to have no impact on the media performance.