

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.....	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

Solution 5

CysteineHCl.....	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.