

## 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 CO<sub>2</sub> 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 <sub>2</sub> HPO <sub>4</sub> .....	1.5 g
DI Water.....	500 ml

### Solution 2

KH <sub>2</sub> PO <sub>4</sub> .....	1.5 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.0 g
NaCl.....	3.0 g
MgSO <sub>4</sub> .....	0.3 g
CaCl <sub>2</sub> .....	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 CO<sub>2</sub> gas. Seal and autoclave at 121°C.

**Solution 6**

Na<sub>2</sub>CO<sub>3</sub>.....6.0 g  
DI Water.....100 ml

Boil briefly under CO<sub>2</sub> gas. Seal and autoclave at 121°C.