

ATCC medium: 988 *Spiroplasma* medium SP-4

PPL0 Broth w/o CV (BD 255420).....11.0 g
Tryptone (BD 211705).....10.0 g
Glucose.....5.0 g
Distilled water.....625.0 ml

Adjust pH to 7.5. Autoclave at 121C for 15 minutes.

Add Aseptically:

Mycoplasma Growth Supplement (CMRL 1066)(10X)*.....50.0 ml
Yeast Extract Solution (see below).....35.0 ml
TC Yeastolate (2% soln.) (BD 255772).....100.0 ml
Fetal bovine serum (heat-inactivated)**.....170.0 ml
Phenol red solution (0.1% soln.).....20.0 ml

Mycoplasma Growth Supplement:

Prepare by adding 1.0 L of water to one bottle of CMRL-1066 powder. Mix to dissolve and filter-sterilize.

*This supplement is now available as ATCC 20-2206: 98.0 g powder and as ATCC 20-2207: 100.0 ml (10x solution).

**Replaceable with newborn calf serum.

Yeast Extract Solution:

Bakers' yeast (live, pressed, starch-free).....250.0 g
Distilled deionized water.....1.0 L

Mix yeast and water; autoclave at 121C for 90 minutes at 15 psi pressure. Allow to stand. Remove supernatant solution. Adjust pH to 6.6-6.8.