ATCC Medium: 0988 Spiroplasma Medium
NOTE: This medium has been reformulated as MD-2611
Check with Biologist to make sure they are getting what they actually need.

REAGENTS:

N/A □

Basal Medium
PPLO Broth w/o Crystal Violet (Difco or eq.) …………… 11 g
Tryptone (Difco or eq.) ……………………………………… 10 g
Agar (if required) ……………………………………………… 15 g
DI Water …………………………………………………… 500 mL

N/A □

Additive Solution
CMRL-1066 Medium*(ATCC 20-2207) … 50 mL
Yeast Extract #3 (ATCC MD-9678 ) ………………………… 60 mL
TC Yeastolate (Difco 5577 or eq.) …………………………. 2 g
Fetal bovine serum (heat inactivate, ATCC 30-2020 SEE NOTE) ……………… 170 mL
0.1% Phenol Red Solution (See below)…………………… 20 mL
Glucose ……………………………………………………… 5 g
DI water …………………………………………………… 200 mL

N/A □

0.1% Phenol Red Solution
Phenol Red………………………………………………… 0.1 g
0.1N NaOH………………………………………………… 20 mL
DI Water…………………………………………………… 80 mL

PROCEDURE:

Steps

N/A □

To make ATCC Medium 0988:
Accurately weigh out reagents for the Basel Medium
Dissolve completely into DI water
Start time: End time:
Adjust to pH 7.3 - 7.4
Autoclave at 121°C for 15 minutes
For Agar, cool to 50-55 °C in a water bath.
Add filter-sterilized Additive Solution to the Basal Medium (see below)
Gently mix.
If necessary, re-adjust pH to 7.35 ± 0.1.
Dispense into appropriate vessel type.

N/A □

To make Additive Solution:
Accurately measure and weigh out reagents
Dissolve completely into DI water
Filter sterilize

N/A □

To make 0.1% Phenol Red Solution:
Accurately weigh out Phenol Red
Dissolve into 0.1N NaOH
Add DI water
Mix well
If necessary, add 6N NaOH dropwise to complete dissolving.

NOTE:

*Custom CMRL Powder in use at ATCC, as of 1/2/03, contains L-glutamine.

**Heat Inactivation of FBS:
Thaw a bottle of Fetal Bovine Serum
Place into the water bath (55°C).
Place a large beaker over the bottle to hold heat
Allow the bottle to remain in the water bath for 1 hour and 15 minutes.
(15 minutes to come to 55°C and 1 hour to heat inactivate).