

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 Basal 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).