

ATCC Medium: 2611 Spiroplasma Medium – Special Modified Formulation

Basal Medium

PPLO Broth w/o Crystal Violet (BD 255420).....	3.5 g
Pancreatic Digest of Casein (Hardy C6530).....	10.0 g
Pancreatic Digest of Gelatin (Hardy C6540).....	5.0 g
Agar (if required).....	15 g
DI Water.....	500 ml

Adjust pH to 7.4 +/- 0.2. Autoclave at 121°C. If agar is used, cool to 50-55 °C in a water bath. Combine the 500 ml of additive solution to the basal medium. Gently mix; if necessary, readjust pH to 7.4 +/- 0.2. Dispense as required.

Additive Solution

10X CMRL 1066 Medium (ATCC 20-2207).....	50.0 ml
Yeast Extract Solution (see below).....	35.0 ml
TC Yeastolate (BD 255772) 10% solution.....	20.0 ml
Fetal bovine serum (see below).....	170.0 ml
Phenol Red Solution 0.1% Solution (see below).....	20.0 ml
Glucose.....	5.0 g
DI Water.....	205 ml

Combine ingredients and filter sterilize through 0.22 microns.

Yeast Extract Solution

Yeast Extract (Hardy C7340).....	15 g
DI Water.....	100 ml

Dissolve yeast extract with constant stirring. Filter once using a crepe fluted filter (VWR 28331-106 or equivalent). Add 35 ml to additive solution.

Fetal Bovine Solution (heat inactivated)

Thaw a bottle of Fetal Bovine Serum (ATCC 30-2020). 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).

Phenol Red Solution

Add Phenol Red powder to 1/5th required volume of 0.1 N NaOH to dissolve. When dissolved, add remaining 4/5th of volume (water). Examine to confirm that phenol red has dissolved. Add additional 6 N NaOH drop by drop, if necessary to complete the dissolving of the phenol red.

Note: CMRL 1066 Medium is made as a 10X solution from custom CMRL Powder (20-2206) manufactured by Sigma. Custom formulation in use at ATCC,

as of 1/2/03, contains L-glutamine

CMRL Solution and FBS, if known to be sterile, may be added directly to the basal medium and omitted from the Additive Solution. This makes filter-sterilization easier.