

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 C6531)	10.0 g
Pancreatic Digest of Gelatin (Hardy C6541)	5.0 g
Agar (if required)	15.0 g
DI Water.....	500.0 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 and 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, see below) 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.....	205.0 mL

Combine ingredients and filter sterilize through 0.22 microns.

Yeast Extract Solution

Yeast Extract (Hardy C7340)	15.0 g
DI Water.....	100.0 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.

10% TC Yeastolate solution

TC Yeastolate (BD 255772).....	10.0 g
DI Water.....	100.0 mL