

ATCC medium: 1796 CRY5 medium

Stock Extract (see below).....500.0 ml
2x Concentrated Medium 1651 (see below).....500.0 ml

Autoclave for 15 minutes at 121C.

Stock Extract:

Cerophyl.....5.0 g
Brown rice.....5.0 g
Yeast Extract (BD 212750).....5.0 g
Dried seaweed.....5.0 g
Distilled water.....500.0 ml

Boil medium for 5 minutes, filter three times through Whatman #1 filter paper while hot, cool, adjust to pH 7.2 and bring volume back to 500 ml with distilled water. The stock extract can be stored at -20C.

2X Concentrated Medium 1651:

Prepare Medium 1651 (see below) at 2X concentration. The final specific gravity should be 1.030.

ATCC Medium 1651:

L-alpha-lecithin.....0.2 g
Proteose Peptone (BD 211684).....10.0 g
Trypticase Peptone (BD 211921).....10.0 g
Ribonucleic Acid from Torula Yeast (Sigma R6625).....1.0 g
Vitamin Solution (see below).....2.0 ml
Artificial Seawater (Aqua-Marin) (see below).....500.0 ml
Distilled water.....500.0 ml

Emulsify L-alpha-lecithin in warm distilled water before adding remaining powdered ingredients. Adjust to pH 7.2. Add vitamin mix and artificial seawater; readjust to pH 7.2, if necessary. Dispense 5.0 ml per 16 x 125 mm screw-capped test tube and autoclave at 121C for 15 minutes.

Artificial Seawater (Aqua-Marin):

Aqua-Marin*.....20.8 g
Distilled water.....500.0 ml

*Note: Aqua-Marin available from: Aquatrol, Inc., 237-H North Euclid Street, Anaheim, CA

Vitamin Solution:

Biotin (dissolve in ethanol, 1 mcg/ml).....	10.0 ml
Folic Acid (Sigma F7876).....	50.0 mg
Nicotinamide.....	50.0 mg
Calcium D-(+)-pantothenate (Sigma T2250).....	100.0 mg
Pyridoxal . HCl.....	50.0 mg
Riboflavin (Sigma R4500).....	50.0 mg
Thiamine . HCl (Sigma T4625).....	150.0 mg
DL-6 Thiocctic acid.....	1.0 mg
Distilled water.....	100.0 ml

Mix components under constant stirring. For long-term storage, dispense in 2 ml aliquots in 16 X 125 mm screw-capped test tubes, flush with nitrogen, and store at -20C.