

ATCC medium: 1873 Seawater microaerophile medium

ATCC Medium 1171 (see below).....250.0 ml
ATCC Medium 1525 (see below).....750.0 ml

Allow ATCC medium 1525 to cool before adding to ATCC medium 1171. Thoroughly suspend the starch and aseptically dispense in 12.0 ml aliquots to 16 X 125 mm screw-capped test tubes.

ATCC medium 1171, TYGM-9 medium:

K₂HPO₄2.8 g
KH₂PO₄0.4 g
Casein Digest (BD 211610).....2.0 g
Yeast Extract (BD 211929).....1.0 g
NaCl.....7.5 g
Gastric mucin.....2.0 g
Distilled water.....970.0 ml

Prepare ingredients in order listed. Autoclave for 15 minutes at 121C. Cool. Aseptically add:

Bovine serum (heat-inactivated)..... 30.0 ml
10% Tween 80 in absolute ethanol (filter-sterilized)... 0.5 ml

Aseptically dispense 8.0 ml per 16 x 125 mm screw-capped test tube. Aseptically add the rice starch solution (see below), 0.15 to 0.40 ml per test tube. The purer the rice starch, the lower the quantity used.

Rice Starch Solution:

Heat-sterilize 0.5 g of rice starch* at 150C for 2 hours. After sterilization and just prior to use, aseptically add 9.5 ml of sterile phosphate buffered saline solution at pH 7.4. Extra rice starch solution should not be kept.

*Rice starch available from Merck KGaA, Frankfurter Strasse 250, 64293 Darmstadt, Germany; product number: 111684. Other brands may not be milled fine enough to support growth of organisms. See: <http://www.merck.de/english/index.htm> for ordering information.

ATCC medium 1525, Seawater 802 medium

Solution 1:

2X Artificial seawater.....500.0 ml

Filter-sterilize.

Solution 2:

Rye grass cerophyll solution (see below).....500.0 ml

When cool, aseptically combine Solutions 1 and 2.

Cerophyll.....2.5 g
Na₂HPO₄0.25 g
Distilled water.....500.0 ml

Add cerophyll to distilled water and boil for 5 minutes. Add 50 ml of distilled water to compensate for evaporation. Filter through Whatman #1 filter paper and add the Na₂HPO₄. Autoclave for 15 minutes at 121C.