ATCC medium: 1865 Axenic *Dimastigella* medium

Sonneborn's Paramecium Medium (ATCC Medium 802, see below).....980.0 ml
Vitamin Solution (see below).....10.0 ml
Heat-killed Bacterial Suspension (see below).....10.0 ml

ATCC Medium 802:
Solution 1, Rye grass Cerophyll:
Cerophyll*.....2.5 g
Distilled water.....1.0 L

Add cerophyll to distilled water and boil for 5 minutes. Add 100 ml distilled water to compensate for evaporation. Filter through Whatman #1 filter paper and add 0.5 g Na_2HPO_4. Autoclave for 15 minutes at 121C.

Solution 2 Agar for Klebsiella pneumoniae:
Agar.....20.0 g
Yeast extract.....4.0 g
Glucose.....0.16 g
Distilled water.....800.0 ml

Dispense in 5.0 ml amounts. Autoclave for 25 minutes at 121C. Slant. Add the bacterium, grown on solution 2, to solution 1 and incubate at 30C for 24 hours prior to inoculation with Paramecium.

*Can be obtained from Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, NY 14692. Dairy Goat Nutrition, P.O. Box 22363, Kansas City, MO 64113 distributes Grass Media Culture which is equivalent. Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178, sells Cereal Leaf Product no. C-7141 which is similar to Cerophyll.

Vitamin Solution:
Calcium D-(+)-pantothenate...0.05 g
Nicotinamide.....0.05 g
Pyridoxal . HCl.....0.05 g
Pyridoxamine . HCl.....0.025 g
Riboflavin.....0.05 g
Folic acid.....0.025 g
Thiamine . HCl.....0.15 g
Biotin.....0.0125 mg
DL-Thioctic acid.....0.5 ml
Distilled water.....100.0 ml

Add 10.0 ml of the Vitamin Solution (the solution will appear cloudy, agitate vigorously before dispensing) to 980 ml of Medium 802. Autoclave for 15 minutes at 121C. Cool and dispense in 10-ml aliquots to T-25 tissue culture flasks. Add 0.1 ml of the Heat-killed Bacterial Suspension to each flask just before inoculation with the protist.

Heat-killed Bacterial Suspension:
Prepare heat-killed Klebsiella pneumoniae subsp. pneumoniae ATCC 27889 in the following manner:
1. Inoculate a loopful of ATCC 27889 from a nutrient agar slant into 5 ml of nutrient broth. Incubate at 35C overnight.
2. Add 0.5 ml of bacterized broth prepared in step 1 to each of ten 1-L Erlenmeyer flasks, each containing 250 ml of Nutrient Broth. Incubate cultures at 35C for 24 hours.
3. Aseptically transfer bacterial suspensions to 500-ml sterilized screw-capped centrifuge bottles. Fill bottles with a maximum of 400 ml. Centrifuge in a refrigerated centrifuge at 5000 rpm for 10 minutes.
4. Decant supernatant and resuspend pellets in Page's Balanced Salt Solution (Medium 1323, see below). Pool all suspensions in a single bottle and centrifuge as in step 3.
5. Discard supernatant and resuspend pellet in Medium 1323 (final volume of cell suspension should be approximately 400 ml).
6. Repeat step 5 twice more.
7. Resuspend the final pellet in less than 100 ml of Medium 1323. Make sure cells are thoroughly suspended.
8. Transfer to a 125 ml screw-capped serum bottle and dilute to a final volume of 100 ml with Medium 1323.
9. Do a serial dilution of the suspension prepared in step 8. Carry dilution out to 10(-9) dilution. Plate 0.1 ml aliquots in triplicate from the 10(-7) - 10(-9) dilution tubes. Place the aliquots in the center of 100 mm petri plates containing nutrient agar and spread evenly over the surfaces with a spread bar. Incubate plates at 35C overnight.
10. Place bottle prepared in step 8 in a 60C water bath so that the liquid level of the water bath is above that of the suspension in the bottle. At 10-minute intervals swirl the bottle. Incubate for a total of 30 minutes. Allow the bottle to cool to room temperature. This treatment should kill all bacterial cells.
11. As a check for viable cells, add 3 drops of the cell suspension prepared in step 10 to the edge of a 100 mm petri plate containing nutrient agar. Hold the plate vertically to allow the drops to move to the opposite edge. Incubate plate at 35C for 48 hours.
12. Determine bacterial cell concentration from plates prepared in step 9.
13. Adjust the concentration of the heat-killed bacteria to 10(10) cells/ml.

ATCC Medium 1323:
Solution 1:
Na_2HPO_4 .....2.84 g
KH_2PO_4 .....2.72 g
Distilled water.....500.0 ml

Autoclave for 20 minutes at 121C.

Solution 2:
$MgSO_4 \cdot 7H_2O$ .....8.0 mg
$CaCl_2 \cdot 2H_2O$ .....8.0 mg
NaCl.....0.240 g
Distilled water.....500.0 mg

Autoclave for 20 minutes at 121C.
Combine solutions 1 and 2 when cooled to room temperature.