

CELL CULTURING PROTOCOL: Daoy (HTB-186; ATCC) – human medulloblastoma cells from a cerebellum

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Cat. No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Atmosphere: air 95%, carbon dioxide (CO₂) 5%.

Temperature: 37.0°C

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended

Medium Renewal: 2—3 times per week.

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

**Eagle's Minimum Essential Medium (EMEM)
Formulation
Cat. No. 30-2003 (ATCC)**

Medium components:

Inorganic Salts (g/liter)

CaCl₂ (anhydrous) 0.20000
MgSO₄ (anhydrous) 0.09767
KCl 0.40000
NaHCO₃ 1.50000
NaCl 6.80000
NaH₂PO₄·H₂O 0.14000

Amino Acids (g/liter)

L-Alanine 0.00890
L-Arginine-HCl 0.12640
L-Asparagine-H₂O 0.01500
L-Aspartic Acid 0.01330
L-Cystine-2HCl 0.03120
L-Glutamic Acid 0.01470
L-Glutamine 0.29200
Glycine 0.00750
L-Histidine-HCl·H₂O 0.04190
L-Isoleucine 0.05250
L-Leucine 0.05250
L-Lysine-HCl 0.07250
L-Methionine 0.01500
L-Phenylalanine 0.03250
L-Proline 0.01150
L-Serine 0.01050
L-Threonine 0.04760
L-Tryptophan 0.01000
L-Tyrosine-2Na·2H₂O 0.05190
L-Valine 0.04680

Vitamins (g/liter)

Choline Chloride 0.00100
Folic Acid 0.00100
myo-Inositol 0.00200
Nicotinamide 0.00100
D-Pantothenic Acid 0.00100
(hemicalcium)
Pyridoxine-HCl 0.00100
Riboflavin 0.00010
Thiamine-HCl 0.00100

Other (g/liter)

D-Glucose 1.00000
Phenol Red, Sodium Salt 0.01000
Sodium Pyruvate 0.11000