

CELL CULTURING PROTOCOL: LN-18 (CRL-10442; ATCC) – human glioblastoma cells from a temporal lobe

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium (Cat. No. 30-2002). 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 37C 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 37C.

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

Medium Renewal: Every 2—3 days.

Preservation:

Freeze medium: Complete growth medium 90%, additional fetal bovine serum 5%, DMSO 5%.

Storage temperature: liquid nitrogen vapor phase

**Dulbecco's Modified Eagle's Medium (DMEM)
Formulation
Cat. No. 30-2002 (ATCC)**

Medium components:

Inorganic Salts (g/liter)

CaCl₂ (anhydrous) 0.20000

Fe(NO₃)₃·9H₂O 0.00010

MgSO₄ (anhydrous) 0.09770

KCl 0.40000

NaHCO₃ 1.50000

NaCl 6.40000

NaH₂PO₄·H₂O 0.12500

Amino Acids (g/liter)

L-Arginine-HCl 0.08400

L-Cystine·2HCl 0.06260

L-Glutamine 0.58400

Glycine 0.03000

L-Histidine-HCl·H₂O 0.04200

L-Isoleucine 0.10500

L-Leucine 0.10500

L-Lysine-HCl 0.14600

L-Methionine 0.03000

L-Phenylalanine 0.06600

L-Serine 0.04200

L-Threonine 0.09500

L-Tryptophan 0.01600

L-Tyrosine·2Na·2H₂O 0.10379

L-Valine 0.09400

Vitamins (g/liter)

Choline Chloride 0.00400

Folic Acid 0.00400

myo-Inositol 0.00720

Nicotinamide 0.00400

D-Pantothenic Acid 0.00400 (hemicalcium)

Pyridoxine-HCl 0.00400

Riboflavin 0.00040

Thiamine-HCl 0.00400

Other (g/liter)

D-Glucose 4.50000

Phenol Red, Sodium Salt 0.01500

Sodium Pyruvate 0.11000