CELL CULTURING PROTOCOL: HCN-1A (CRL-10442; ATCC) – human cortical neurons

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%.

Temperature: 37.0℃

Growth Conditions: The growth medium must be adjusted to pH 7,s35 prior to filtration

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 which 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. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended

Medium Renewal: 1—2 times per week

Preservation:

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

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)
$CaCl_2$ (anhydrous) 0.20000
$Fe(NO_3)_3 \cdot 9H_2O$ 0.00010
$MgSO_4$ (anhydrous) 0.09770
KCl 0.40000
$NaHCO_3$ 1.50000
NaCl 6.40000
$NaH_2PO_4 \cdot H_2O$ 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·H_2O 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·2H_2O 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