



HD Community Biorepository
Cell Line Description and Propagation Instructions



CHDI#	CHDI-90000419
Coriell Ref Number	CH00286
Cell Line Name	PC12, Htt-SW2-Q73, rdm, 1-3144, human, clone 29 Alias: PC12-SW2-FLHtt73-CL29
Description	PC12 cells inducibly expressing a full length human Huntingtin (Htt) containing 73 polyglutamine repeats translated from a random codon. rdm: x/CAG/CAA/x
Host Cell line name, species and tissue source	PC12, rat, pheochromocytoma of adrenal gland
Engineered DNA construct, include reference	Htt-SW2-Q73, rdm, 1-3144, human (CHDI-90000428) Alias: SW2-FLHttQ73
Induction system utilized	RheoSwitch
Immortalization method used if any	Not Applicable
Complete growth medium with recommended manufacturer	Kaighn's Modification of Ham's F-12 (ATCC # 30-2004) 15% Horse Serum (Gibco # 16050-122) 2.5% FBS (Hyclone # SH30071) 1% Pen/Strep: Hyclone cat # SV30010 0.2 mg/ml active G418 (Calbiochem cat # 345810) 0.2 mg/ml Zeocin (Invitrogen cat # 46-0072) *Requires collagen IV substrate - see attachment
Is it being cultured in the presence of antibiotics?	Yes-see above
Temperature	37°C
Atmosphere	5% CO ₂ , humidified
Subcultivation ratio	1:3
Max tolerable cell density or confluency	90%
Medium renewal	3-4 days
Appearance/Morphology, etc	Small round and clumpy
Growth Properties (adherent, etc)	Adherent but require collagen IV substrate
Freeze medium	50% Growth medium + 50% Cryoprotective medium (Lonza 12-132A)
Storage temperature	Liquid Nitrogen vapor
Species and tissue of origin, geographical source of isolation, and any known associated hazards (HIV, EBV etc)	rat, pheochromocytoma of adrenal gland (ATCC CRL-1721)
Recommended biosafety level for working with this strain	1
Miscellaneous Background Information, specific notes and supporting data	Sigma Col IV Cat#C5533 or Fluka Biochemika cat # 27663

PC12 lines expressing Full-length WT and Mutant Huntingtin

I. Cell Subculture and Maintenance Protocol

- a. Cells grown in collagen IV coated flasks and either replenished with fresh medium or subcultured at 1:2 – 1:3 every 3-4 days.
- b. Medium
 - i) Kaighn's Modification of Ham's F-12 (ATCC # 30-2004, lot 3000608)
 - ii) 15% Horse Serum (Gibco # 16050-122, lot 675855)
 - iii) 2.5% FBS (PAA # A15-201, lot A20106-7030)
 - iv) 1% Pen/Strep: Hyclone cat # SV30010, lot # JTG32463
 - v) 0.1 mg/ml active G418 (GIBCO cat # 10131-035, lot # 449728)
 - vi) 0.1 mg/ml Zeocin (Invivogen cat # ant-zn-5, lot 30-16-zl)
- c. Collagen IV: Sigma cat # C5533, lot 087K3780

II. Collagen IV Coating Protocol

- a. Make a 0.5 mg/ml solution of Collagen IV by dissolving 5mg Collagen IV in 10 ml of 0.25% acetic acid in HBSS
- b. Incubate overnight at 4°C with occasional mixing
- c. Next day dilute collagen IV solution to 0.1mg/ml with 0.25% acetic acid in HBSS
- d. To coat flasks, add 3 ml (T25), 5.0 ml (T75), or 10.0 ml (T150) of collagen IV solution
- e. Incubate for 30 minutes at room temperature
- f. Remove the excess collagen IV
- g. Incubate flasks overnight in hood
- h. Store at 4°C
- i. To coat 384 well plates, add 16ul of the 0.1 mg/ml Collagen IV solution
- j. Incubate for 10 min at room temperature
- k. Dump off excess collagen IV solution
- l. Dry overnight in hood
- m. Store at 4°C

III. Seeding/Induction Protocol

- a. Remove media from T-75 flask and wash with 3 ml Trypsin-EDTA (Mediatech, Manassas VA).
- b. Incubate with 3 ml Trypsin-EDTA for 15 minutes at 37° C until cells begin to detach
- c. Wash cells from flask with 3 ml complete medium and transfer to a 15 ml conical tube. Count live cells using trypan blue exclusion and seed 1×10^3 cells in 100µl of medium per well of a collagen IV-coated 96-well plate (ViewPlate, PerkinElmer, Shelton, CT).
- d. For induction, cells are treated with 10µM, 25µM, or 50µM of Ponasterone A (Axxora, LLC, San Diego, CA) in 10µl of medium/well.
- e. For neurite outgrowth and differentiation, cells are treated with 100 ng/ml or 200 ng/ml of Nerve Growth Factor (Sigma).