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<th><strong>CHDI#</strong></th>
<th>CHDI-90000419</th>
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<td><strong>Coriell Ref Number</strong></td>
<td><strong>CH00286</strong></td>
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| **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% CO2, 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 4ºC 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 4ºC
   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 4ºC

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 37º 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 X 103 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).