<table>
<thead>
<tr>
<th>CHDI#</th>
<th>CHDI-90000072</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coriell Ref #</td>
<td>CH00096</td>
</tr>
<tr>
<td>Cell Line Name</td>
<td>STHdhQ7/Q111</td>
</tr>
</tbody>
</table>

**Description**

Striatal derived cell line from a knock in transgenic mouse containing heterozygous Huntingtin (HTT) loci with a humanized Exon 1 with 111 polyglutamine repeats and the other with a humanized Exon 1 with 7 polyglutamine repeats.

**Host Cell line name, species and tissue source**

E14 striatal precursor cell lines, from mouse E14 homozygous Htt Q7/Q111 embryo, striatum.

**Engineered DNA construct, include reference**

tsA58/U19 large T antigen

**Induction system utilized**

Permissive temperature 33°C

**Immortalization method used**

Infection of a defective retrovirus transducing the tsA58/U19 large T antigen, selected with G418 resistant colonies at permissive 33°C.

**Complete growth medium**

- DMEM 440ml (80%)
- FBS 50ml (10%)
- Penicillin/streptomycin 5ml (100x)
- G418 stock (40mg/ml) 5ml (100x)

**Is it being cultured in the presence of antibiotics?**

Original submissions of cells by Dr. MacDonald were grown in the presence of Pen-Strep and G418 (0.4mg/ml). The cells you are receiving are cultivated in the absence of these antibiotics.

**Temperature**

33°C

**Atmosphere**

5% CO2, humidified

**Subcultivation ratio**

1:4 to 1:16

**Medium renewal**

3-4 days

**Appearance/Morphology, etc**

Fibroblast look with small branch

**Growth Properties (adherent, etc)**

Adherent monolayer

**Freeze medium**

Recovery™ - Cell Culture Freezing Medium (source: GIBCO #12648)

**Storage temperature**

-150°C (Nitrogen tank)

**Miscellaneous Background Information, specific notes and supporting data**

Growth Condition for Marcy MacDonald’s S7Hdh Cell Lines

The following protocol was provided by Marcy MacDonald (MGH) and CombinatoRx

1. MATERIALS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Vendor</th>
<th>Cat#</th>
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</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>Gibco</td>
<td>11965-118</td>
</tr>
<tr>
<td>Fetal Bovine Serum (FBS)</td>
<td>Gibco</td>
<td></td>
</tr>
<tr>
<td>G418</td>
<td>Gibco</td>
<td>11811-031</td>
</tr>
<tr>
<td>Penicillin/Streptomycin (Pen/Strep)</td>
<td>Gibco</td>
<td></td>
</tr>
<tr>
<td>0.25% trypsin-EDTA</td>
<td>Gibco</td>
<td></td>
</tr>
</tbody>
</table>

Cell line source: Dr. Marcy MacDonald at MGH

<table>
<thead>
<tr>
<th>CHDI#</th>
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<th>DESCRIPTION</th>
</tr>
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<tbody>
<tr>
<td>CHDI-9000071</td>
<td>ST HdhQ111/Q111</td>
<td>Striatal neuronal cell line derived from an Hdh111/111 knock in mouse</td>
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<tr>
<td>CHDI-9000072</td>
<td>ST HdhQ7/Q111</td>
<td>Striatal neuronal cell line derived from an Hdh7/111 heterozygous knock in mouse</td>
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<tr>
<td>CHDI-9000073</td>
<td>ST HdhQ7/Q7</td>
<td>Striatal neuronal cell line derived from an Hdh7/7 “wild-type” knock in mouse</td>
</tr>
</tbody>
</table>

Incubator culture conditions: 5% CO₂, 33°C
Equipment: microscope, hemacytometer

2. PROCEDURES:

Make a complete medium with G418 (recipe is for 500mL):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>DMEM</td>
<td>440ml</td>
<td>88%</td>
</tr>
<tr>
<td>FBS</td>
<td>50ml</td>
<td>10%</td>
</tr>
<tr>
<td>Pen/Strep</td>
<td>5ml</td>
<td>100x</td>
</tr>
<tr>
<td>G418 stock</td>
<td>5ml</td>
<td>100x</td>
</tr>
</tbody>
</table>

Complete media without G418 was used when thawing new vial of cells

1. For thawing new vial of cells, warm up a T75 flask with ~20mL of complete media without G418 in 5% CO₂, 33°C incubator.
2. Take cells from liquid nitrogen tank, thaw in 33°C water bath (1~2min) and quickly add 1ml warm complete media without G418 in it.
3. Transfer the whole content into the T75 flask and mix gently.
4. Incubate at 33°C incubator for overnight.
5. Change to fresh complete media without G418.
6. Incubate at 33°C incubator for 5~7 days till it is over 70% confluent, media could be changed at 2~3 days.
7. For splitting cells, warm up medium and 0.25% trypsin-EDTA to 33°C.
8. Aspirate old medium and wash once with 1x PBS.
9. Add 3 ml 0.25% trypsin-EDTA to the T75 flask and sit for 5~6 min, rock gently around 2min~3min, be sure all cells are dissociated.
10. Add 7 ml of the medium to the flask to inactivate trypsin.
11. Pipette cells several times to make sure that cells are well separated
12. Cells could be split 1:2 ~ 1:16 into new flasks. Incubate plates at 33°C till it is over 70% confluence
STHdh striatal -derived cell differentiation protocols
Provided by Marcy MacDonald

Both protocols should be optimized for cell number, coated/uncoated plastic surface, as for primary neurons, Fetal Bovine Serum (FBS) lot and incubation temperature.

Fetal Bovine Serum (FBS) is important and different lots of FBS will support different growth and differentiation characteristics.

The response to the DOPA cocktail is very rapid and cells appear morphologically distinct within 4-6 h. Once projections are apparent cells may be retained on the dish for 24-48 hours, then will detach, though how long the cells adhere to the dish depends on the particular subclone, history of passage (lower passage numbers give a more robust and stable response) and FBS lot, cell number and substrate.

Experiments can also be performed at the non-permissive temperature for the Ts SV40 large T antigen (39ºC), though this tends to give a very rapid response, with rapid loss of cells from the dish after projections are apparent.

- Culture cells at 33ºC in Complete DMEM (DMEM + 10%FBS +Pen/Strep/Glu G418) O/N on culture dishes
- Wash cells with PBS and add “DOPA” Cocktail in complete DMEM or “DOPA” Cocktail in DMEM without FBS

**Complete DMEM**
DMEM, 500 ml
Pen/Strep/Glu, 5 ml of 100X solution
G418, 5 ml of 40 mg/ml
10% Fetal Bovine Serum (FBS)

**Serum Free Medium (SFM)**
DMEM, 500 ml
Pen/Strep/Glu, 5 ml of 100X solution
G418, 5 ml of 40 mg/ml

**DOPAMINE (DOPA) Cocktail - reagents -Sigma**
α-FGF (10 ng/ml)
IBMX (250 µM) [3-isobutyl-1-methylxanthine]
TPA (200 nM) [Phorbol 12-myristate 13-acetate]
Forskolin (50µM)
Dopamine (20µM; 10µM, 5µM): depends on response

**Note:** Prepare the “DOPA” cocktail, with fresh stock solutions for the reagents, especially for forskolin.

**Complete DMEM + DOPA Cocktail - reagents -Sigma**
Filter with 0.2 µm filter

**Serum Free Medium (SFM) + DOPA Cocktail - reagents Sigma**
Filter with 0.2 µm filter