HD Community Biorepository
Cell Line Description and Propagation Instructions

CHDI#	CHDI-90000071
Coriell Ref #	**CH00095**
Cell Line Name	ST*Hdh*Q111/Q111
Description	Striatal derived cell line from a knock in transgenic mouse containing homozygous Huntingtin (HTT) loci with a humanized Exon 1 with 111 polyglutamine repeats
Host Cell line name, species and tissue source	E14 striatal precursor cell lines, from mouse E14 homozygous Htt Q111/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	33ºC
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	See attached Protocols: Growth Conditions for S*T*Hdh Cell Lines & STHdh striatal – derived cell differentiation

Growth Condition for Marcy MacDonald's *ST*Hdh Cell Lines

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

1. MATERIALS

Reagent	Vendor	Cat#
DMEM	Gibco	11965-118
Fetal Bovine Serum (FBS)	Gibco	
G418	Gibco	11811-031
Penicillin/Streptomycin (Pen/Strep)	Gibco	
0.25% trypsin-EDTA	Gibco	

Cell line source: Dr. Marcy MacDonald at MGH

CHDI#	NAME	DESCRIPTION
CHDI-9000071	ST HdhQ111/Q111	Striatal neuronal cell line derived from an Hdh111/111 knock in mouse
CHDI-9000072	ST HdhQ7/Q111	Striatal neuronal cell line derived from an Hdh7/111 heterozygous knock in mouse
CHDI-9000073	ST HdhQ7/Q7	Striatal neuronal cell line derived from an Hdh7/7 "wild-type" knock in mouse

Incubator culture conditions: 5% CO_2, 33°C

Equipment: microscope, hemacytometer

2. PROCEDURES:

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

DMEM	440ml	(88%)
FBS	50ml	(10%)
Pen/Strep	5ml	(100x)
G418 stock (40mg/ml)	5ml	(100x)

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% CO_2, 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 (39°C), though this tends to give a very rapid response, with rapid loss of cells from the dish after projections are apparent.

- Culture cells at 33°C 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 Bovie 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

a-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