



HD Community Biorepository
Cell Line Description and Propagation Instructions



CHDI#	CHDI-90000066
Coriell Ref #	CH00066
Cell Line Name	ST14A HTT-Q15, 1-548, HUMAN
Description	Striatal cell line derived from rat embryonic day 14 (E14) expressing a Huntingtin fragment (1-548) containing 15 polyglutamine repeats
Complete growth medium	Refer to additional content regarding medium for passaging of ST14A cells
Is it being cultured in the presence of antibiotics?	Original submission of ST14A cells were grown in the presence of Pen-Strep. The cells you are receiving are cultivated in the absence of Pen-Strep.
Temperature	33°C
Atmosphere	5% CO ₂
Storage temperature	-150°C (Liquid Nitrogen tank)
Miscellaneous Background Information, specific notes and supporting data	Please review entire content information on ST14A cells

INFORMATIONS ON ST14A CELLS

Department of Pharmacological Sciences and
Center of Excellence on Neurodegenerative Diseases
University of Milano
Via Balzaretto 9, 20133 MI-ITALY
Tel +39-0250318333, +39-0250318349-353
Fax +39-0250318284

Contact persons:
marta.valenza@unimi.it
elena.cattaneo@unimi.it

Content:

Medium for normal passaging	page 2
Medium for cell differentiation	page 3
How to handle cells	page 5
Transfection	page 7
Infection	page 9
Immunocytochemistry	page 11
Protocol for quiescence and differentiation	page 12
How to test for the effects of exogenous genes	page 12
Doxy-inducible ST14A cells	page 13
Published information on ST14A cells	page 14

MEDIUM FOR NORMAL PASSAGING OF ST14A CELLS

DMEM + 10% FCS

DMEM (high glucose) (500 ml Euroclone #ECB7501L)
Add previously sterilized:
L-glutamine 5 ml (-20°C SIGMA#G7513, 200mM)
Pen-Strep 6ml (-20°C GIBCO #600-5075AE, 100,000 U/ml))
50 ml FCS (-20°C) previously heat inactivated 1h 56°C and filtered 0,22µm.
This is regular growth medium.

HBSS' w/o Ca⁺⁺ Mg⁺⁺

Add to distilled water:
HBSS w/o Ca Mg 9,5 g/l (4°C) SIGMA #H2387
NAHCO₃ 3.7 g/l (RT)
Hepes 3.9 g/l (RT)
Na-piruvate 0,11g/l(4°C)
Pen-Strep 12 ml/l (-20°C GIBCO #600-5075AE)
Filter 0.22 µm.
This is used to rinse cells before trypsinization.

MEDIUM FOR CELL DIFFERENTIATION

Medium for cell differentiation (Mix, see Ehrlich et al, 2001)

To be prepared in SFM (*)

Final concentrations

50 μ M Forskolin SIGMA #F6886
250 μ M IBMX SIGMA #I7018
200 nM TPA SIGMA #P8139
10 μ M dopamine SIGMA #H8502
10 ng/ml aFGF Promega G5061

Forskolin (MW 410,5)

To study activation adenilato cyclase.

10 mg into 1 ml DMSO (24,3 mM). Store at - 20°C into aliquotes.

Add 2 μ l/ml

IBMX (MW 222,2)

Phosphodiesterase inhibitor.

50 mg into 5,5 ml EtOH (40mM). Store at - 20°C into aliquotes.

Add 6 μ l/ml

TPA (MW 616,8)

Tetradecanoilphorbolacetate-PMA, promoter NO, it activates PKC.

5 mg into 1,62 ml DMSO (5mM). Store at - 20°C into aliquotes.

You have to take 3 μ l of this solution and to dilute them into 300 μ l of normal medium (50 μ M).

Add 4 μ l/ml of 50 μ M solution.

Dopamine (MW 189,6)

50 mg into 5,2ml (5mM). Store at - 20°C into aliquotes.

Add 2 μ l/10ml

AcidicFGF

Solve into 100 μ l of PBS+BSA 1% previously sterilized. Stock solution 100ng/ μ l.

Store at - 20°C into aliquotes of 10 μ l each. You have to dilute one of this stock solution by adding 90 μ l SFM or PBS (10ng/ μ l).

Add 1 μ l/ml

To prepare 10 ml of Mix add to **SFM***:

20 μ l forskolin 24,3 mM

60 μ l IBMX 40mM

40 μ l TPA 50 μ M

2 μ l dopamine 5mM

10 μ l aFGF 10ng/ml

Medium for cell differentiation, SFM *

F12 : DMEM (high glucose) (GIBCO #21331-020)

Add previously sterilized:

L-glutamine 5 ml (-20°C SIGMA#G7513, 200mM)

Pen-Strep 6ml (-20°C GIBCO #600-5075AE)

N2 1x (100X -20°C GIBCO #17502-048)

HOW TO HANDLE CELLS

Passaging of ST14A

Cells grow easily and nicely at 33°C in 5% CO₂.

Split the cells routinely when the plate is 90% confluent.

Cells can grow on regular tissue culture-treated plastic w/o coating.

Regular passaging of the cells is performed at 1:8 dilution maximum.

Avoid higher dilution.

Avoid cells overgrowth (growth is however contact-inhibited)

We always use the same batch of serum for all passages (we use stocks of serum for 4-5 years)

Trypsinizing cells

Trypsinization is performed according to standard procedures. Use Trypsin-EDTA (GIBCO # 610-5400 diluted in HBSS w/o Ca⁺⁺Mg⁺⁺ to 1X).

1. Aspirate medium from the culture dish.

2. Rinse cells twice with HBSS w/o calcium and magnesium. The monolayer should be covered with HBSS.

3. Pour off medium. Add 3 ml of 1X Trypsin to each 10 cm² dish.

4. Monitor the trypsinization process at room temperature under microscope.

5. When cells are dislodged, immediately transfer them in a 50 ml plastic tube with 5 times volume of DMEM + 10% FCS

6. Spin 1000 rpm 5'.

7. Aspirate medium, flick the cells pellet and resuspend in fresh DMEM + 10% FCS

8. Seed aliquotes to new plates with complete medium.

Cryopreservation

Freezing is performed according to standard procedures.

1. Prepare a solution of 5% DMSO in FCS. Store it at - 20°C.

2. Rinse the dishes 90% confluent with HBSS twice.

3. Add 3 ml of trypsin 1X per 10 cm² plate. Check cells under microscope

4. Transfer the cells detached from the plate into a 50 ml tube containing 5 times volume of DMEM + 10% FCS.

5. Centrifuge 1000 rpm 5'. Aspirate off almost all medium

6. Flick the cells pellet.

7. Add 1 ml 5% DMSO/FCS to cells from 1 plate. Mix by pipetting

8. As quickly as possible, transfer cells to dry ice and, once frozen, to liquid nitrogen.

Thawing

1. Take an aliquote out of the N₂ container. Rapidly warm up the tube in the incubator

2. Transfer cells to a 50 ml plastic tube in which you have previously added 10 ml DMEM + 10% FCS

3. Spin cells down at 1000 rpm 5'.

4. Aspirate off almost all the medium

5. Flick the cell pellet, add 10 ml DMEM + 10% FCS.

6. Plate the cells.

INFECTION OF ST14A CELLS

(a) Retroviral vector

We have used various retroviral vector (also EGFP tagged versions). One easy to use is the pLXSP which confers the resistance to the puromycin (Cattaneo et al., JBC, 1996).

(b) Packaging cell line

We use RetroPack PT67 Cell Line (Clontech #K1060-D) that contains the Moloney murine leukemia virus MoMuLV genes *gag*, *pol* while the *env*. is 10A1-derived. The cells can therefore infect rodent and humans. P2 culture facilities are required. Alternatively we use Ecopack 293 (Clontech #C3200-1). This is an ecotrophic virus and can infect only rodent.

Perform the transfection of the selected packaging cells using Calcium Phosphate.

It is possible to use the viral supernatant obtained from the transiently transfected cells, but to reach a good infection efficiency we recommend to work with viral supernatant obtained from stably transfected cells. You can select clones and test them for viral production or obtain a pool of stably transfected cells.

Once you have the stably transfected packaging cells (either single clones or pooled clones), store them in liquid nitrogen. Avoid to keep them in culture.

1. When you need to perform an infection, thaw a tube of packaging and plate them into two 100mm dishes.
2. Let cells grow to 90% confluency.
3. The evening before infection, replace the medium with 6ml of fresh medium in a 100 mm dish (regular DMEM with 10%FCS).
4. The day after, collect the medium and filter it using 0,45 μ M sterile filters. Add it to ST14A cells (prepared as described below) and leave it for 8-10 hours.
5. We normally perform 3 cycles of infections to cover the 24-30 hours. Freshly collected viral supernatant should be used at each time (one can obtain it from the same dish of retropack or from parallel dishes).
6. Discard the packaging cells that have been used.

One can also prepare a large volume of viral supernatant and freeze it (-80°C) and use it when necessary. Freezing, however, decreases infection efficiency.

(c) Infection

1. Plate 1x10⁶ ST14A cells into 100 mm dishes in DMEM + 10%FCS and incubate them at 33°C overnight in 5% CO₂.
2. Next evening replace the medium with medium carrying the viral particles. Add 6 ml of medium to each dish (if you plate the ST14A cells in smaller dishes you can use less viral medium). Incubate cells at 33°C for 8-10 hours.
3. Next day, in the morning, remove the viral containing medium and replace it with new virus containing medium. You can add polybrene to a final concentration of 8 micrograms/ml (we do not normally use it).
4. In the evening, remove the virus containing medium and replaced with new virus containing medium.
5. Next day, in the morning, remove the virus containing medium and replace with new fresh medium.
6. The following day, apply the antibiotic selection (puromycin 3 micrograms/ml -for the pLXSP vector-).
7. Change the medium every 4-5 days with antibiotic selection.

8. Select colonies

notes

During infection all liquids and items in contact with virus are to be treated as biohazardous. Expose them with bleach, work with gloves.

IC ON GLASS COVERSLEIPS

Plate the cells on round glass coverslip coated with polyornithine (*) or laminin.

These coverslips fit into a 24 well plate (i.e. 12 mm size).

Expose cells at the desired conditions.

When ready, wash two times with PBS and fix cells with paraformaldehyde 4% (PFA 4%) in phosphate buffer, pH 7,4

Rinse 3x PBS and leave in PBS Na Azide 0,1% at 4°C, if to be stored. Otherwise proceed as follows (you can remove the coverslip from the plate and rinse it by dipping it in PBS or add PBS to the well).

Blot off PBS on kleenex, place the coverslip on Eppendorf cap face up. (eppendorf cap can be attached face up to a tray).

If antigen is internal, use blocking solution with TRITON (PBS + 1% FCS +0,4% TRITON) to loosen up membrane 10' RT (all cytoskeletal antigens are internal).

Rinse 3x PBS.

Apply 1° antibody in PBS (50 µl/coverslip) 1h RT or O/N 4°C. Make sure coverslips don't dry out during overnight incubation with antibody.

Rinse 3x PBS.

Apply 2° antibody in PBS 1h RT.

Rinse 3x PBS.

Mount face down on glass slide with a drop of immunomount (Permafluor).

Poly-Ornithine *

Sigma #P6355

100X Poly-ornithine stock : 150 mg PORN / 100 ml ddW

Keep at -20°C into 10 ml aliquotes.

-Dilute 1X (10 ml + 900 ml ddw).

-Filter 0,22 µm.

-Add enough porn to cover the plate.

-Leave 20' at least at RT

-Rinse 2X PBS.

PROTOCOL FOR QUIESCENCE AND CELL “DIFFERENTIATION”

Cells normally grow at 33°C in medium containing FCS. Attempts at differentiating the cells may consider:

1. shifting of the cells to 39°C in DMEM+FCS. This reduces cell division and causes the disappearance of the T-Antigene (see Cattaneo et al., 1994; Cattaneo and Conti, 1998). The cells will slowly continue to divide, however, since in serum they will turn mostly into astrocytes.
2. shifting of the cells to 39°C in SFM (see protocol for solutions) evokes little morphological changes and robust apoptotic cell death within 2-4 days (see Rigamonti et al., 2000).
3. shifting of the cells to SFM at 33°C is also possible. However, cells slow down cell division and become flat (see Cattaneo and Conti, 1998)
4. shifting of the cells to the “Mix” (see protocol for solutions) at 33°C nicely evokes morphological differentiation and action potentials (see Ehrlich et al., 2001). This procedure produces the best type of differentiation, however many cells undergo cell death.

None of these conditions, however, faithfully reproduce a normal physiological programme of differentiation (which is not unexpected). However if one wants to look at selected aspects of such a programme, the cells can turn very useful. For example since with protocol 2 cells die with a precise time course, one can select this protocol to look for the effect of a transgene in preventing cell death. Or, genes influencing positively cell division or differentiation can be identified by exposing cells to 39°C. These strategies have been successfully applied in our lab and the data have been subsequently confirmed in transfected primary cells or animal models. Since stable subclones of parental cells can be easily generated, ST14A cells have also been successfully used for gene delivery and gene function studies.

HOW TO TEST FOR THE EFFECTS OF EXOGENOUS GENES ON CELL SURVIVAL, PROLIFERATION, DIFFERENTIATION

Any of the conditions above may be used. Those conditions induce precise and reproducible changes in the behaviour of the cells (in term of their survival, proliferation, differentiation). Importantly, if one wants to engineer the cells to express a foreign gene we recommend the gene to be delivered via retroviral transduction and not by simple transfection. This allows to generate subclones that more stably express the transgene even at the non permissive temperature (or after transplantation). In this way one can test for the effects of the transgene at very long time points following the shift of temperature (even after 20 days!) since the transgene will not be silenced.

Doxy-INDUCIBLE ST14A CELL LINES

We have recently generated a variant of ST14A cells where expression of a transgene can be induced after addition of doxycycline to the cells. The system is based on the TetOn gene expression system. One first round of engineering has produced the stably engineered rtTA ST14A subclone expressing the transactivator. In this subclone the activity of the transactivator is higher regulated by doxy-cycline. This engineered cells can be used to express any gene cloned under the TRE Tetracycline Responsive Element (see detailed infos in Sipione et al., 2002 Hum Mol Genet).

Starting from this rtTA ST14A subclone, we have generated cells expressing the huntingtin gene regulated by doxycycline: we found that expression of the transgene is strictly regulated in a time and dose dependent manner upon exposure to the inducer.

Cells grow routinely at 33° in DMEM+10% FCS using a batch of serum certified as Tetracycline free (Clontech). When you wish to induce transgene expression, plate the cells in DMEM + 10% FCS and, after adhesion, expose the cell to doxycycline (SIGMA) (1 μ g/ml) for the desired time.

SELECTED PUBLISHED INFORMATIONS ON ST14A CELLS

1. ST14A cells were derived from the rat embryonic striatum through retroviral transduction of a temperature sensitive SV40 Large TAg oncogene. ST14A cells have properties of CNS progenitor cells including (i) expression of Nestin, an antigen specifically found in immature CNS progenitor cells (ii) response to specific growth factors and (iii) the capability to differentiate, to some extent, in vitro, into MAP2 positive cells (a marker for mature neurons) (Cattaneo, *J. Neurosci. Res.*, 1998). In addition, we reported that (iv) ST14A cells express antigens specific of medium spiny striatal neurons such as DARPP-32, dopamine receptors, Adenosine A2A receptors (Ehrlich, *Exp. Neurology*, 2001; Varani, *Faseb J.*, 2001) and that (v) action potentials can be measured in the cells following exposure to a differentiating Mix (*Exp. Neurology*, 2001). Although striatal antigens are expressed by the cells, the fact that ST14A cells express both markers of the immature and mature cell state indicates that cell immortalization has altered, to some extent, the properties of the cells.
2. The presence of a conditional oncogene allows to regulate the growth of the cells in vitro and in vivo. ST14A cells are suitable for cell transplantation studies since the temperature of inactivation of the conditional oncogene corresponds to the body temperature of rodents. We reported that ST14A cells can be transplanted into the embryonic (Cattaneo et al., *Dev. Brain Res.* 1994) and adult brain (Lundberg et al., *Exp. Neurology* 1997) where a percentage of the cells differentiate into neurons (2-4%) and glia (20%). No tumor formation has been noted in any of the transplantations performed (up to two years post-transplantation).
3. ST14A cells can be genetically engineered to express genes of interest for biological studies (Cattaneo et al. *Journal of Biol. Chem.* 1996). By using the LipoFectamine based method (a procedure we also optimized for human CNS stem cells, Cattaneo et al. *Molecular Brain Research* 1996), transient transfection efficiency with the lacZ plasmid reaches 30-40%. For stable transgene expression we use retroviruses, allowing the gene of interest to be stably expressed also at the non-permissive temperature. Recently, ST14A cells were used to model Huntington's Disease and to assess the function of huntingtin (Rigamonti et al., *J. Neuroscience* 2000; Zuccato et al., *Science* 2001; Zuccato et al., *Nat. Genetics* 2003). The immortalized ST14A cells and primary cells were also used to investigate the role of Shc adaptors in neural stem cell biology (Conti et al., *PNAS* 1997; *Nature Neuroscience* 2001).
4. ST14A cells have been employed for gene therapy approaches in animal models of diseases. The cells have been genetically engineered to produce IL4-for antitumoral gene therapy purposes (Benedetti et al., *Human Gene Therapy* 1997; Benedetti et al., *Nature Medicine*, 2000) or Galc (Torchiana et al., *Neuroreport* 1998).
5. Earlier attempts at using ST14A cells to express genes of interest under the control of a regulatable promoter (such as that of the Tet system) have been performed. Transient regulation of a permanently expressed LacZ gene under the control of the tet system (Tet Off) has been achieved in vivo after transplantation of the engineered ST14A cells and following injection of the Tetracyclin analogue into

the animal (Corti et al. *Neuroreport* 1996). More recently, derivatives of the ST14A cells were produced that stably expressed mutant huntingtin under the Tet control system (Tet On). The cells were used for gene chips studies (Sipione, *Human Mol Genetics*, 2002) and in Valenza et al., *J. Neuroscience* 2005.

6. Evidence also indicates that ST14A cells express intracellular signalling molecules similar to those found in their in vivo counterparts (Conti et al. *PNAS* 1997; De Fraja et al., *J. Neurosci. Res.* 1998; Conti et al., *Nat.Neurosci.*, 2001). This indicates that the cells may be suitable for studies related to growth factors signalling during cell differentiation.