# Product Specification Sheet

| | |
|---|---|
| **Product Name** | **Human iPS Cells (inducible Ngn2)** |
| **Description** | ALSTEM offers human iPS cell line with inducible expression of Ngn2 by doxycycline (Dox). This cell line is generated from pre-made in footprint-free human iPS cells with the inducible Ngn2 that has been stably integrated into the human AAVS1 "Safe Harbor" site. It provides a convenient means to differentiate human iPS cells to excitatory neurons rapidly and efficiently. The integration at "Safe Harbor" sites ensure safe and reliable expression of Ngn2 when Dox is supplied. |
| **Catalog Number** | iP11NA |
| **Size** | $5x10^5$ cells/vial |
| **Shipping** | Dry ice |
| **Storage and Stability** | Store in gas phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed. |
| **Quality Control** | Human iPS cells (inducible Ngn2) were grown in feeder-free conditions with mTeSR1 medium. Each lot of hiPSCs is tested for growth and viability following recovery from cryopreservation. In addition, each lot is tested for expression of TRA-1-60 and OCT4. |
| **Safety Precaution** | **ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask always be worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force. |
| **Restricted Use** | For Research Use Only. Not for use in diagnostic or therapeutic procedures. |

**ALSTEM, INC**
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com

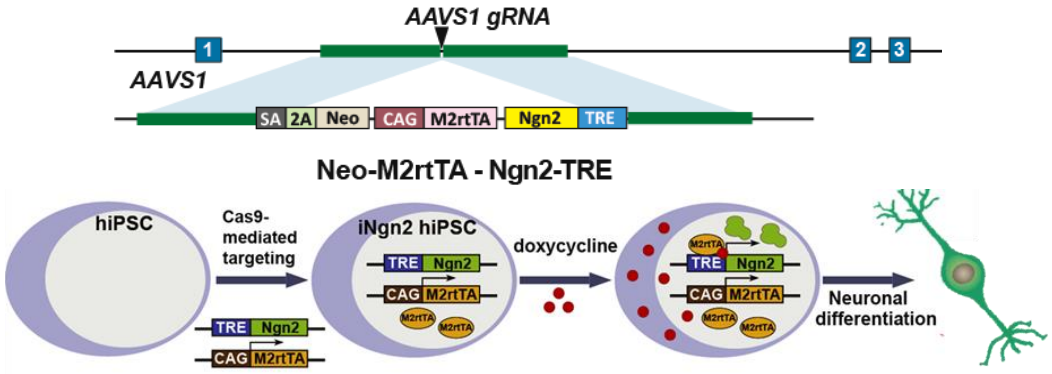
# Protocol

## Human iPS Cell Culture and Genome Editing

### OVERVIEW

Neurogenin (Ngn) 2 is a basic helix-loop-helix (bHLH) transcription factor that promotes direct differentiation of pluripotent stem cells to functional excitatory neurons. AAVS1 (also known as PPP1R12C) locus supports robust and sustained transgene expression. Precise insertion of tetracycline inducible Ngn2 into human iPS cells at AAVS1 site could generate a stable iPSC line for rapid neuronal differentiation upon induction.

The ALSTEM scientists developed a highly responsive doxycycline inducible Ngn2-expressing human iPS cell line, which is ideal for *in vitro* rapid neuronal differentiation. ALSTEM's doxycycline inducible Ngn2-expressing iPS cell line (cat# iP11NA) is produced from pre-made in footprint-free human iPS cells by integrating the all-in-one inducible Ngn2 cassette into the human AAVS1 "Safe Harbor" site.




This all-in-one cassette consists of both inducible Ngn2 and constitutive M2rtTA that has been inserted into the same allele at the AAVS1 locus by gene editing through HDR pathway, while the other allele of AAVS1 locus remains intact. Upon induction, Ngn2 is expressed and the iPS cells will be directly converted into excitatory neurons.

## PROCEDURE

## I. Feeder-free human iPSC culture conditions

**Preparation of feeder-free medium**

1. Thaw mTeSR1 5X Supplement (Cat.no. 05850, STEMCELL Technologies) at room temperature or overnight at 4°C.
2. Add the 100 mL of thawed 5X Supplement to 400 mL Basal Medium for a total volume of 500 mL aseptically. Mix well and filter through a 0.22 µm, low-protein binding filter, if desired.
3. Aliquot into appropriate volumes according to usage and store the aliquots at 4°C.

**ALSTEM, INC**
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com

**Coating plates with Matrigel**

Matrigel (Cat.no. 354277, BD) should be aliquoted and stored at -80°C for long-term use.

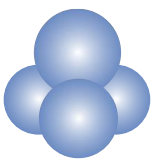
1. Thaw Matrigel on ice until liquid. Dilute Matrigel 1:50 with pre-chilled KO DMEM/F12.
2. Immediately use the diluted Matrigel solution to coat tissue culture-treated plates. For a 6-well plate, use 1 mL of diluted Matrigel solution per well, and swirl the plate to spread the Matrigel solution evenly across the surface.
3. Let the coated plate stand for 1 h at 37°C or overnight at 4°C. If plate has been stored at 4°C, allow the plate to incubate at 37°C for at least 30 minutes before removing the Matrigel solution.

**Thawing cryopreserved human iPS cells (inducible Ngn2)**

1. Quickly thaw the human iPS cells (inducible Ngn2) in a 37°C waterbath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol to sterilize.
2. Transfer the contents of the cryovial to a 15 mL conical tube. Add 5 mL warm mTeSR1 dropwise to the tube, gently mixing as the medium is added.
3. Centrifuge cells at 200 *x g* for 5 minutes at room temperature.
4. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the cell pellet in 2 mL mTeSR1 with 10 µM ROCK inhibitor, taking care to maintain the cells as small cell clumps.
5. Remove the Matrigel solution from a coated tissue culture 6-well plate. Transfer the medium containing the cell clumps to the Matrigel coated 6-well plate.
6. Place the plate into the 37°C incubator and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% $CO_2$ and 95% humidity.
7. Change medium daily. Check for undifferentiated colonies that are ready to passage when colonies are big enough (approximately 7-10 days after thawing).

**Passaging human iPS cells (inducible Ngn2) grown under feeder-free conditions**

1. Use a microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.
2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the human iPS cell culture and rinse with DPBS (2 mL/well).
4. Add 1 mL per well of EZStem Enzyme-Free Stem Cell Dissociation Solution (cat. no. M100, ALSTEM), and incubate at 37°C for 2-3 minutes. Or add 0.5 mL per well of accutase (Cat.no. SCR005, Millipore, diluted 1:2 with DPBS before use), and incubate at 37°C for 1-2 minutes.
5. Remove EZStem Enzyme-Free Stem Cell Dissociation Solution or accutase, gently rinse each well 2 - 3 times with 2 mL of DMEM/F-12 per well and transfer the detached cell aggregates to a 15 mL conical tube.
6. Add 2 mL/well mTeSR1 and scrape colonies off with a cell lifter. Transfer the detached cell aggregates to the above 15 mL conical tube.
7. Rinse the well with an additional 2 mL mTeSR1 to collect any remaining aggregates. Add the rinse to the 15 mL tube.
8. Centrifuge the 15 mL tube containing the aggregates at 200 x *g* for 5 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 5 µM ROCK inhibitor by gently pipetting and ensure that cells are maintained as aggregates.



**ALSTEM, INC**
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com

10. Plate the human iPS cell aggregates with mTeSR1 in a new plate coated with Matrigel. (Remove Matrigel solution before plating). *If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:3 to 1:6 ratios.*
11. Place the plate into the 37°C incubator and move the plate quickly back and forth and side to side to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% $CO_2$ and 95% humidity.
12. Change medium daily, omitting the ROCK inhibitor after 24 hours.

**Cryopreserving human iPS cells (inducible Ngn2)**

1. Prepare EZStem Freezing Medium (Cat.no. M050, ALSTEM) or mTeSR1 medium containing 10% DMSO and 10 µM ROCK inhibitor, and keep on ice.
2. Perform steps 1-8 from **Passaging human iPS cells grown under feeder-free conditions.**
3. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
4. Gently resuspend the pellet in freezing medium, taking care to leave the clumps larger than that would normally be done for passaging.
5. Transfer 1 mL of clumps in freezing medium into each labeled cryogenic vial.
6. Place vials into a freezing container and place the container at -80°C overnight.
7. Transfer to a liquid nitrogen tank the next day.

## II. Neuronal induction of human iPSCs (inducible Ngn2)

### Cell culture media

**N2 medium**: To make 500 mL: 480 mL DMEM/F12, 5 ml N2 supplement (100X), 5 ml GlutaMAX (100X), 5 ml NEAA (100X) and 5 ml Pen/Strep (100X). Filter with a 0.22 µm filter and store at 4°C for up to one month.
**Glia medium**: To make 500 mL: 465 mL DMEM, 5 mL GlutaMAX (100X), 25 mL FBS (final concentration 5% vol/vol) and 5 ml Pen/Strep (100X). Filter with a 0.22 µm filter and store at 4°C for up to one month.
**Growth medium**: To make 500 mL: 455 mL Neurobasal Medium, 5 mL GlutaMAX (100X), 10 mL B-27 Supplement (50X), 25 mL 5% FBS (final concentration 5% vol/vol) and 5 ml Pen/Strep (100X). Filter with a 0.22 µm filter and store at 4°C for up to one month.

**Neuron induction**

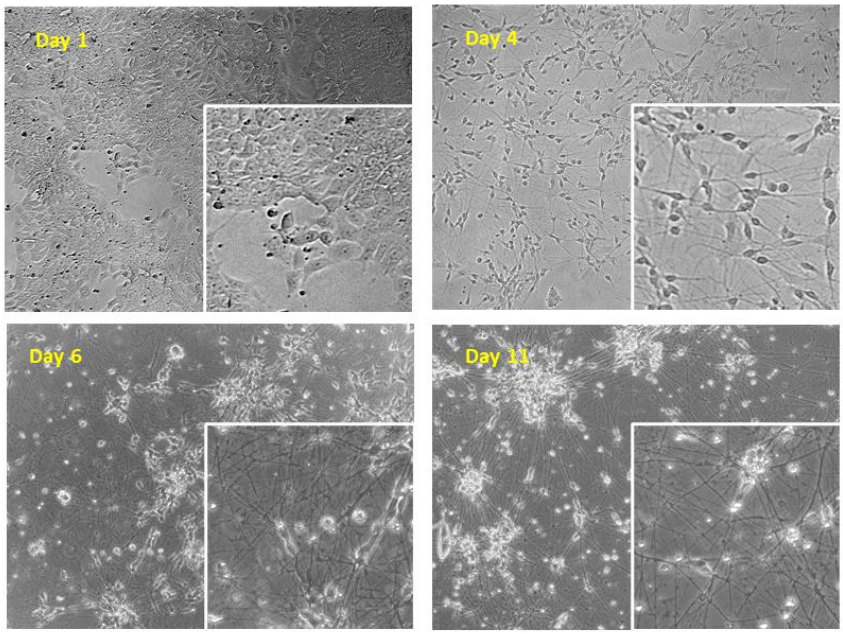
**(Day 0) Plate human iPS cells (inducible Ngn2)**

1. Use a microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.
2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the human iPS cell culture and rinse with DPBS (2 ml/well).
4. Add 0.5 ml per well of Accutase (Millipore, Cat. # SCR005, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1 min.
5. Remove Accutase, and gently rinse each well 2-3 times with 2 ml of DMEM/F-12 per well to wash away remaining enzymes.
6. Add 2 ml/well mTeSR1 and scrape colonies off with a cell scraper.

7. Transfer the detached cell aggregates to a 15 ml conical tube and rinse the well with an additional 2 ml mTeSR1 to collect any remaining aggregates. Add the rinse to the 15 ml tube.
8. Centrifuge the 15 ml tube containing the aggregates at 200x g for 5 min at room temperature.
9. Aspirate the supernatant. Resuspend pellet in mTeSR1 containing 5 µM ROCK inhibitor by gently pipetting and ensure that cells are maintained as small aggregates. Count the cells.
10. Plate $5x10^4$ – $1x10^5$ cells/well of human iPS cells with mTeSR1 onto one well of a 6-well plate coated with Matrigel (remove Matrigel solution before plating).
11. Place the plate into the 37°C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C with 5% CO2 and 95% humidity.

**(Day 1-30) Transduction and induction**
12. On day 1, remove old medium from the culture and add 2 ml mTeSR1.
13. On Day 2, remove old medium from the culture and add 2 ml N2 media with 2ug/ml Doxycycline.
14. On Day 3 and Day 5, change the media with 2 ml N2 media with 2µg/ml Doxycycline.
15. On Day 6, put coverslip into 24-well plate, add 0.3 ml Matrigel and incubate 1 hour at 37°C.
16. Dissociate induced neurons with Accutase, adjust the induced neuron number to $4x10^5$ and mouse glial cells to 5 $x10^4$, and mix the cells with 0.5 ml Growth Medium with Doxycycline (2µg/ml) onto one coverslip.
17. On Day 8-15, change half of the media every other day using Growth Medium with Doxycycline (2µg/ml) and Ara-C (4µM).
18. On Day 16, remove Doxycycline from the Growth Medium and add BDNF (20ng/ml).
19. On Day 19-30, change half of the media (0.25 ml) every 3-4 days using Growth Medium with Ara-C (4µM) and BDNF (20ng/ml).

**ALSTEM, INC**
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com




**Upon doxycycline treatment, inducible Ngn2 iPS cells differentiate to neurons. (A)** In the absence of doxycycline, the iPS cells retain the pluripotent state, however, the cell morphology changes even at 1 day after Dox treatment. **(B) 4** days after Dox treatment, iPS cells are starting to differentiate to neurons. **(C)** 4 days after Dox treatment, majority of cells have converted to neurons with extensive growth of axons and dendrites. **(D)** 11 days after Dox treatment, these cells have typical neuronal morphology.

## IMPORTANT NOTICE

Store the vials at vapor phase of liquid nitrogen immediately upon receipt.

## WARNING

**Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures which are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.**

**ALSTEM, INC**
2600 Hilltop Drive, BLDG B, STE C328, Richmond, CA 94806
Tel: (510) 708-0096 Fax: (866) 605-8766
www.alstembio.com info@alstembio.com