

AUG 04, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.x54v9p8b4g3e/v1

External link:
<https://doi.org/10.7554/eLife.72328>

Protocol Citation: Jiuchun Zhang, Harper JW 2023. Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.x54v9p8b4g3e/v1>

MANUSCRIPT CITATION: Eapen VV, Swarup S, Hoyer MJ, Paulo JA, Harper JW, Quantitative proteomics reveals the selectivity of ubiquitin-binding autophagy receptors in the turnover of damaged lysosomes by lysophagy. eLife doi: [10.7554/eLife.72328](https://doi.org/10.7554/eLife.72328)

Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells

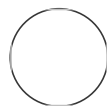
Forked from [Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells](#)

In 1 collection

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ABSTRACT

This protocol is about neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells.

ATTACHMENTS

[Neural_differentiation_of_AAVS1-TRE3G-NGN2-pluripotent_stem_cells.pdf](#)

SAFETY WARNINGS



For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

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Protocol status: Working

Created: Jun 23, 2023

Last Modified: Aug 04, 2023

PROTOCOL integer ID: 83946

Keywords: ASAPCRN

BEFORE START INSTRUCTIONS

Prepare ND1 Medium and ND2 Medium:

ND1 Medium

DMEM/F12

N2 (100x) 1x

BDNF 10 ng/ml

NT3 10 ng/ml

NEAA (100X) 1x

Laminin 0.2 µg/ml

Doxycycline 2 µg/ml

ND2 Medium

Neurobasal medium

B27 (50x) 1x

Glutamax (100x) 1x

BDNF 10 ng/ml

NT3 10 ng/ml

Doxycycline 2 µg/ml

Note

- * Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).
- * Cells can be split from Day 5 to Day 7 (or even earlier-not tested yet).

Day 0:

- 1 Treat H9 AAVS1-TRE3G-NGN2 cells with Accutase and plate the dissociated cells in matrigel-coated 6-well plates (2×10^5 cells/well) in **ND1 Medium** supplemented with Y27632 (**10** 10 micromolar (µM)).

Day 1:

- 2 Replace the medium with **ND1 Medium**.

Day 2:

- 3 Replace the medium with **ND2 Medium**.

Day 4:

- 4 Exchange 50% of the medium from each well.

Day 6:

- 5 Treat the cells with Accutase and replate the dissociated cells in matrigel-coated 6-well plates (2×10^5 cells/well) in **ND2 Medium**.

Day 8 and thereafter:

- 6 Exchange 50% of the medium from each well every other day.

Note

- * Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).
- * Cells can be split from Day 5 to Day 7 (or even earlier-not tested yet).