

Neural differentiation
of AAVS1-TRE3G-NGN2
pluripotent stem cells

Aug 12, 2021

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pluripotent stem cellsJiuchun Zhang¹, Harper JW¹¹Harvard Medical School

1 Works for me

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ABSTRACT

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

ATTACHMENTS

Neural_differentiation_of_A
AVS1-TRE3G-NGN2-
pluripotent_stem_cells.pdf

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OWNERSHIP HISTORY

Feb 09, 2021  Julia Rossmann **protocols.io**Feb 15, 2021  Felix Kraus

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SAFETY WARNINGS

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

BEFORE STARTING

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

- * Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).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 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 replat the dissociated cells in matrigel-coated 6-well plates ($2-4 \times 10^5$ cells/well) in **ND2 Medium**.

Day 8 and thereafter:

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

* Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).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).