Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells

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Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells

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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).

Protocol status: Working
We use this protocol and it's working

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- * Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).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- * Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).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