

Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells

1. Day 0: Treat 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 μ M).

ND1 Medium:

DMEM/F12	
N2 (100x)	1x
BDNF	10 ng/ml
NT3	10 ng/ml
NEAA(100X)	1x
Laminin	0.2 μ g/ml
Doxycycline	2 μ g/ml

2. Day 1: Replace the medium with **ND1 Medium**.
3. Day 2: Replace the medium with **ND2 Medium**.

ND2 Medium

Neurobasal medium	
B27 (50x)	1x
Glutamax (100x)	1x
BDNF	10 ng/ml
NT3	10 ng/ml
Doxycycline	2 μ g/ml

4. Day 4: Exchange 50% of the medium from each well.
5. Day 6: 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**.
6. Day 8 and thereafter: 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* Doxycycline can be withdrawn on Day 10 (or even earlier-not tested yet).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).