Neuro2a cell DNA plasmid transfection (PolyJet)

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Abstract

Transfection of Neuro2a cells with PolyJet

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Materials

DMEM, High Glucose 11965-092 by Life Technologies

Fetal Bovine Serum, qualified 10437-028 by Life Technologies

PolyJet™ DNA In Vitro Tranfection Reagent SL100688 by Signagen

Protocol

Step 1.
Vendor Recommnded Amounts for Different Culture Vessels

Culture Dish	Culture Medium (mL)	Plasmid (ug)	Diluent (mL)	PolyJet (uL)
48-well	0.3	0.25	2 x 0.015	0.75
12-well	0.75	0.75	2 x 0.038	2.25
6-well	1.0	1.0	2 x 0.05	3.0
35 mm dish	1.0	1.0	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	5.0	2 x 0.25	15.0
T75 flask	8.0	9.8 - 18.0	2 x 0.40	27.0 - 54.0
250 mL flask	18	25 - 50	2 x 0.8	75.0 - 150.0

Step 2.
Split cells 18 - 24hrs prior to transfection

Step 3.
Cells should be at 30 - 80% confluence depending on experiment.

If I plan to collect cells 3 days later, I will transfect at 30% confluence. If I will collect them 1 day later, then I will transfect 80% confluence

Step 4.
For a 6-well plate:

Replace media with 1.0mL fresh culture media 30 - 45 minutes prior to transfection

Step 5.
For each well:

Dilute 2.0 ug DNA in 50mL high glucose DMEM
(12.0ug in 300uL for 6-well plate)

Step 6.
For each well:

Dilute 3.0 uL PolyJet into 50uL high glucose DMEM

(18.0uL in 300uL per 6-well plate)

Step 7.
Add PolyJet/DMEM mixture to DNA/DMEM mixture in 1:1 proportion

Step 8.
Incubate DNA/PolyJet mixture at RT for 15 minutes

Step 9.
Add 100uL DNA/PolyJet mixture to each well

Step 10.
Incubate cells with DNA/PolyJect mixture for 6 - 18hrs

Step 11.
Replace media with fresh culture media