ProMab Biotechnologies, Inc.

Protocol

Important Guidelines for Transfection

OVERVIEW

Cells should be plated 18 to 24 hours prior to transfection so that the cell density reaches 70~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 2 hours before transfection.

TRANSFECTION PROCEDURE

The following protocol is given for transfection in a 24-well plate, refer to **Table 1** for transfection in other culture formats.

1) For each well, add 0.5 ml of normal growth medium (antibiotic does not influence the result) freshly 2 hours before transfection.
2) For each well, dilute 0.5 µg of DNA in 50 µl of DMEM without serum, and Mix gently.
3) Add 1.5 µl of NanoFect reagent (cat.# NF100, ProMab Biotechnologies) into another tube with 50 µl of DMEM without serum, and Mix gently.
4) Add NanoFect/DMEM into DNA/DMEM solution. Mix by vortexing for 5 to 10 seconds.
5) Incubate for ~15 minutes at room temperature to allow for NanoFect/DNA complexes self-assembly.
6) Add the 100 µl NanoFect/DNA mix drop-wise to the cells in each well and homogenize by gently swirling the plate.
7) Return the plates to the cell culture incubator.
8) Check transfection efficiency 24 to 48 hours post transfection.

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Culture Dish Surface	Area (cm^2)	Cell Number	Medium Volume (ml)	Plasmid(μg)	NanoFect (μl)	Diluent Volume (μl)
96-Well	0.3	$1\text{-}1.5x10^4$	0.1	0.1	0.3	10
48-Well	1	$2.5\text{-}5x10^4$	0.25	0.25	0.75	20
24-Well	2	$0.5\text{-}1x10^5$	0.5	0.5	1.5	50
12-Well	4	$1\text{-}2x10^5$	1	1	3	100
6-Well/35 mm	9.5	$2\text{-}4x10^5$	2	2.5	7.55	200
60 mm/T25	28	$5\text{-}10x10^5$	5	6-8	15-24	300
100 mm/T75	79	$1.5\text{-}3x10^6$	10	15-20	35-45	500
150 mm/T150	153	$5\text{-}9x10^6$	20	25-30	60-80	1000

Table 1. Recommended Amounts for Different Culture

Note:

For different cell types, the optimal ratio of NanoFect (μL): DNA (μg) is around 3:1. We recommend the NanoFect (μL):DNA (μg) ratio of 2:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity, however the amount of NanoFect may be adjusted from 2 to 4 μl per μg of DNA depending on the cell line to be transfected. To ensure the optimal size of NanoFect/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and NanoFect Reagent.