

# Protoco

## **Important** Guidelines for Transfection

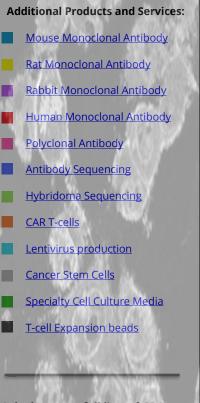
#### **OVERVIEW**

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  $\mu$ l 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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## Protocol

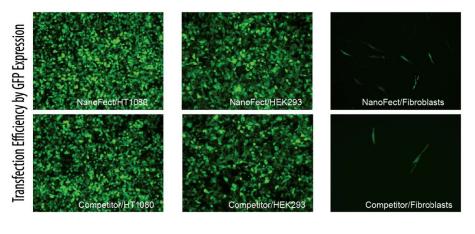
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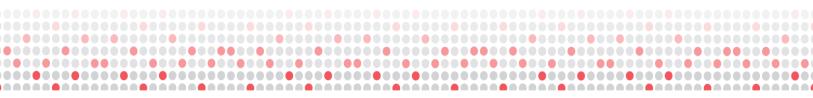
Culture Dish Surface	Area (cm²)	Cell Number	Medium Volume (ml)	Plasmid(µg)	<u>NanoFect</u> (µI)	Diluent Volume (µl)
96-Well	0.3	1-1.5x10 <sup>4</sup>	0.1	0.1	0.3	10
48-Well	1	2.5-5x10 <sup>4</sup>	0.25	0.25	0.75	20
24-Well	2	0.5-1x10 <sup>5</sup>	0.5	0.5	1.5	50
12-Well	4	1-2x10 <sup>5</sup>	1	1	3	100
6-Well/35 mm	9.5	2-4x10 <sup>5</sup>	2	2.5	7.55	200
60 mm/T25	28	5-10x10 <sup>5</sup>	5	6-8	15-24	300
100 mm/T75	79	1.5-3x10 <sup>6</sup>	10	15-20	35-45	500
150 mm/T150	153	5-9x10 <sup>6</sup>	20	25-30	60-80	1000

#### **Table 1. Recommended Amounts for Different Culture**

Note:

For different cell types, the optimal ratio of NanoFect ( $\mu$ L): DNA ( $\mu$ g) is around 3:1. We recommend the NanoFect ( $\mu$ L):DNA ( $\mu$ g) 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  $\mu$ l per  $\mu$ g 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.





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