TOOLSmoothFect Transfection Reagent

Cat. no.: NFT-KA00 / NFT-KA00-10

Product Size:

NFT-KA00 1 mL

NFT-KA00-10 10 x 1 mL

Storage: Store at 4 °C for 6 months.

Introduction

TOOLSmoothFect transfection reagent can be used for transfecting DNA, shRNA, and siRNA. No medium changes are required because TOOLSmoothFect transfection reagent works in the presence of antibiotics and serum. TOOLSmoothFect is associated with an easy-to-use protocol that involves rapid, one-step incubation for 15 min before direct application to target cells; therefore, it is adequately suitable for high-throughput transfection experiments.

Protocol

For high transfection efficiency and relatively low toxicity, transfect cells at high density. Approximately 50%–80% confluence is recommended.

Cells should be plated 18 to 24 h before transfection to ensure that the cell density reaches 50%–80% confluence at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 2 h before transfection. The following protocol is established for transfection in a 24-well plate; refer to Table 1 for transfection in other culture formats.

1. For each well, freshly add 0.5 mL of normal growth medium (antibiotics do not influence the result) 2 h before transfection.
2. For each well, dilute 0.5 μg of DNA in 50 μL of DMEM without serum in a tube and mix gently.
3. Add 1 μL of TOOLSmoothFect to another tube with 50 μL of DMEM without serum and mix gently.
4. Add TOOLSmoothFect/DMEM to DNA/DMEM solution. Mix by vortexing for 5–10 s.
5. Incubate for approximately 15 min at room temperature to allow self-assembly of TOOLSmoothFect/DNA complexes.
6. Add 100 μL of TOOLSmoothFect/DNA mix dropwise 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 24–48 h after transfection.

Table 1. Recommended amounts for different cultures

Culture Dish Surface	Area (cm^2)	Cell Number	Medium Volume (ml)	Plasmid (μg)	TOOLSoothFect (μl)	Diluent Volume (μl)
96-well	0.3	1-1.5x10^4	0.1	0.1	0.3	10
48-well	1	2.5-5x10^4	0.25	0.25	0.75	20
24-well	2	0.5-1x10^5	0.5	0.5	1.5	50
12-well	4	1-2x10^5	1	1	3	100
6-Well/35 mm	9.5	2-4x10^5	2	2.5	7.5	200
60 mm/T25	28	5-10x10^5	5	6-8	15-24	300
100 mm/T75	79	1.5-3x10^6	10	15-20	40-60	500
150 mm/T150	153	5-9x10^6	20	25-40	65-120	1000

Note: For different cell types, the optimal TOOLSmoothFect (μL)-to-DNA (μg) ratio is approximately 3:1. We recommend a TOOLSmoothFect (μL)-to-DNA (μg) ratio of 2:1 as the starting point, which typically yields satisfactory transfection efficiency with invisible cytotoxicity. However, the amount of TOOLSmoothFect could be adjusted from 2 to 4 μL per μg of DNA depending on the cell line to be transfected. Moreover, to ensure the optimal size of TOOLSmoothFect–DNA complex particles, we recommend using serum-free DMEM with high glucose to dilute DNA and TOOLSmoothFect.

The product is for research only, not for diagnostic or clinical use.