For life science research only. Not for use in diagnostic procedures.

TransFecting Reagent

Cat.#: T3010
Size: 2x 100uL ; 5x 100uL; 10x100uL (1mL). With 1xPBS (1mL)
Storage: 2-3 months at 2-8°C. 12-15 months at -20°C.

TransFecting Reagent is a newly formulated DNA and RNA transfection reagent. It has very little cytotoxicity but provides superior transfection efficiency. TransFecting has been proven to be able to deliver DNA or RNA into various established mammalian cell lines and primary cells to express protein or rescue recombinant viruses such as adenovirus or adeno-associated virus (AAV), lentivirus et al.

Key features for TransFecting Reagent

1. **1 ml is generally sufficient for transfection of 500 µg DNA into cells.**
2. **Very low cytotoxicity and higher transfection efficiency.**
3. **TransFecting /DNA or RNA/ siRNA complexes can be added directly into cells culture medium in the presence of serum and/or antibiotics. The presence of serum (10%) and antibiotics usually do not have inhibitory effect on transfection efficiency.**
4. **For protein expression, it does not need to remove complexes or change or add medium following transfection.**

General Protocol

Step I. Cell Seeding:

One day (18 to 24 hours) prior to transfection, sufficient cells are seeded so that the cell density reaches to the optimal 40-90% confluency at the time of transfection.

Step II.

1. **Preparation of TransFecting/DNA or RNA Complex. The optimal ratio of TransFecting (µl):DNA or RNA (µg) is 2:1. (We recommend this ratio of 2:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. User may increase this ratio for desirable transfection efficiency).**
2. **Dilute 2 µl of TransFecting Reagent in 50 µl of dilution buffer, such as serum-free DMEM, RPMI-1640, F17, or PBS (without Ca^{2+} and Mg^{2+}). Gently pipette up and down 3-6 times to mix well.**
3. **Dilute 1 µg of DNA or RNA in 50 µl of dilution buffer in a second tube. Gently pipette up and down 3-6 times to mix well.**
4. **Add the diluted DNA or RNA immediately to the diluted TransFecting solution. Immediately pipette up and down 3-6 times or vortex briefly to mix well.**
5. **Incubate for 15-20 minutes at room temperature to allow TransFecting/DNA or RNA complexes to form. (TransFecting Reagent is slightly cloudy. Formation of TransFecting/DNA or RNA will make solution cloudy).**
6. **Add the resultant TransFecting/DNA or RNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate. Transfected cells are incubated at 37 °C with 5% CO_2.**

Step III. For rescuing recombinant viruses, TransFecting complex must be removed by replacing medium with complete medium 5 hours after transfection.

Table. Recommended Amounts for Different Culture Formats (The amounts of DNA recommended are based on HEK293 cells. For transfection of other cells, especially primary cells, we suggest user to optimize the protocol by increasing the amounts of DNA and the ratio of TransFecting Reagent: DNA (for example 3:1 or 4:1 et al.)

1. Adherent Cell Culture:

Culture vessel	Surf. area per well	Number of Cells (Approximately)	Medium Volume (ml) Per well	Plasmid DNA (µg) Per well	Dilution Volume (µl) Per well	TransFecting Reagent (µl) Per well
96 well plate	0.3 cm^2	$1x10^4$	0.1	0.15	2x 15	0.3
24 well plate	2 cm^2	$5x10^4$	0.5	0.6	2x 30	1.2
12 well plate	4 cm^2	$10x10^4$	1	1.2	2x 60	2.4
6 well plate	10 cm^2	$20x10^4$	2	3	2x 150	6
60 mm dish	20 cm^2	$40x10^4$	5	6	2x 300	12
10 cm dish	60 cm^2	$100x10^4$	10	18	2x 900	36

2. Suspension Cell Culture:

Culture vessel	Surf. area per well	Number of Cells (Approximately)	Medium Volume (ml) Per well	Plasmid DNA (µg) Per well	Dilution Volume (µl) Per well	TransFecting Reagent (µl) Per well
96 well plate	0.3 cm^2	$0.5x10^5$	0.1	0.15	2x 15	0.3
24 well plate	2 cm^2	$2x10^5$	0.5	0.6	2x 30	1.2
12 well plate	4 cm^2	$4x10^5$	1	1.2	2x 60	2.4
6 well plate	10 cm^2	$8x10^5$	2	3	2x 150	6
60 mm dish	20 cm^2	$2x10^6$	5	6	2x 300	12
10 cm dish	60 cm^2	$6x10^6$	10	18	2x 900	36

Precautions and Disclaimer: This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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