

Ver.: 01A

User Bulletin

Lifetech Transfection Reagent

Lifetech Transfection Reagent utilizes LifeTech's proprietary formulation for the transfection of DNA and RNA into eukaryotic cells. Lifetech Transfection Reagent is suitable for many cell types and providing the highly efficiency and low cellular-toxicity.

Transient transfection of adherent cells

For optimal transfection conditions with Lifetech

Transfection Reagent, the cells should be 90-95% confluent. Typically, for transfection in 24-well plates, 5×10^4 to 1×10^5 cells are seeded per well, 24 hours before transfection. For other culture formats, see Table 1.

Shipping & Storage

Lifetech Transfection Reagent is stable for 12 months at 4°C.

Culture Vessel	Number of cells to seed	Vol. of Medium per vessel(ml)	Amount of Plasmid DNA (µg)	Volume of Transfection Reagent (µl)	Total vol. of DNA/reagent complex (µl)
1well/96well	1×10^4 - 1.7×10^4	0.1	0.05~0.2	0.15~0.4	10
1well/24well	5×10^4 - 1.0×10^5	0.5	0.2~1.0	0.6~2.0	30
1well/6well	2×10^5 - 4×10^5	2.0	1.0~3.0	3.0~9.0	120
35mm	2×10^5 - 4×10^5	2.0	1.0~3.0	3.0~9.0	120
60mm	4×10^5 - 8×10^5	5.0	3.0~5.0	6.0~15.0	300
10cm	1×10^6 - 6×10^6	10.0	5.0~10.0	15.0~30.0	600

Table 1: Number of cells to seed before the day of transfection and transfection mix preparation for different cell culture formats.

Culture Vessel	Number of cells to seed	Vol. of Medium per vessel(ml)	Amount of Plasmid DNA (µg)	Volume of Transfection Reagent (µl)	Total vol. of DNA/reagent complex (µl)
1well/96well	2×10^4 - 5×10^4	0.05~0.2	0.15~0.4	0.05~0.2	10
1well/24well	1×10^5 - 2×10^5	0.2~1.0	0.6~2.0	0.2~1.0	30
1well/6well	2×10^5 - 5×10^5	1.0~3.0	3.0~9.0	1.0~3.0	120
35mm	5×10^5 - 2×10^6	1.0~3.0	3.0~9.0	1.0~3.0	120
60mm	2×10^6 - 5×10^6	3.0~5.0	6.0~15.0	3.0~5.0	300
10cm	5×10^6 - 1×10^7	5.0~10.0	15.0~30.0	5.0~10.0	600

Table 2: A Guideline for seeding suspension cells prior to transfection in different culture formats recommended.

Transfection procedure

The following protocol is given for transfection in 24-well plates. Use 1 μ l of Lifetech Transfection Reagent and 1 μ g of DNA per well as follows. See table1 or 2 for other culture vessel formats.

1. Preparation of Lifetech Working Transfection Reagent: Dilute 1 μ l Lifetech Transfection Reagent with 30 μ l serum-free medium (without antibiotics), and then mix by gentle pipetting or vortexing for one second. Incubate the working transfection reagent for 5 minutes at room temperature.

Note: avoiding the Lifetech Transfection Reagent to contact the plastic tube surface

2. Add the 1 μ g plasmid DNA into the Lifetech Working Transfection Reagent and mix by gentle pipetting

3. Incubate the mixture of DNA and Lifetech Transfection Reagent solution at room temperature for 15 minutes. For some cell line the incubation may be up to 30 minutes.

4. Add DNA/Lifetech Transfection Reagent complex to cells in each well and mix by gentle shaking plate, Incubate cells at 37°C in CO₂ incubator for 18-48 hrs.

Transfection with siRNA

The following protocol is given for transfection in 24-well plates.

1. Preparation of Lifetech Working Transfection Reagent: Dilute 1 μ l Lifetech Transfection Reagent with 30 μ l serum-free medium (without antibiotics), and then mix by gentle pipetting or vortexing for one second. Incubate the working transfection reagent for 5 minutes at room temperature.

2. Add 100 pmol siRNA into the Lifetech Working Transfection Reagent and mix by gentle pipetting

3. Incubate the mixture of siRNA and Lifetech Transfection Reagent solution at room temperature for 15 minutes.

4. Add siRNA/ Lifetech Transfection Reagent complex to cells in each well and mix by gentle shaking plate, Incubate cells at 37°C in CO₂ incubator for 24-72 hrs.

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