HP-TransFecting Reagent Kit

HP-TransFecting Reagent is a newly formulated DNA and RNA transfection reagent. It has very little cytotoxicity but its transfection efficiency is 2-10 folds more than transfection reagent commonly used in the lab. HP-TransFecting has been proven to be able to deliver DNA or RNA into various established mammalian cell lines and primary cells to express protein.

Cat.#: T3030
Size: Enhancer (100uL); HP-Transfecting reagent (100uL); dilution buffer (15 mL)
Storage: -20°C.

Key features for HP-TransFecting Reagent

1. **1 ml is generally sufficient for transfection of 1000 µg DNA into cells.**
2. **Very little cytotoxicity and high transfection efficiency.**
3. **Enhancer/ DNA or RNA/HP-TransFecting triple complexes can be added directly into cells culture medium in the presence of serum and 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 (14 to 20 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.

Preparation the triple complex: Enhancer+DNA/RNA + HP-TransFecting.

1. **Dilute 1 µl of Enhancer in 40 ul dilution buffer in a clean tube. Gently pipette up and down 3-6 times to mix well.**
2. Dilute 1 µg of DNA or RNA in 40 µl dilution buffer in a second tube. Gently pipette up and down 3-6 times to mix well.
3. Immediately mix the diluted DNA or RNA to the diluted Enhancer (Total 80uL volume). Pipette up and down 3-6 times or vortex briefly to mix well.
4. Incubate for **10 minutes** at room temperature to prepare Enhancer/DNA or RNA complex.
5. Dilute 1 µl of HP-TransFecting in 40 µl dilution buffer in a clean tube. Gently pipette up and down 3-6 times to mix well.
6. Add the diluted HP-TransFecting to Enhancer/DNA or RNA complex (Total 120uL volume). Immediately pipette up and down 3-6 times or vortex briefly to mix well.
7. Incubate for **15 minutes** at room temperature to allow Enhancer//DNA or RNA/HP- TransFecting triple complexes to form.
8. Add the resultant Enhancer//DNA or RNA/HP-TransFecting **triple complexes 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.

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 Enhancer or HP-TransFecting Reagent.

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	3x 10	0.3
24 well plate	2 cm^2	$5x10^4$	0.5	0.6	3x 24	1.2
12 well plate	4 cm^2	$10x10^4$	1	1.2	3x 48	2.4
6 well plate	10 cm^2	$20x10^4$	2	3	3x 120	6
60 mm dish	20 cm^2	$40x10^4$	5	6	3x 240	12
10 cm dish	60 cm^2	$100x10^4$	10	18	3x 540	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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