Instruction of HighGene Transfection Reagent

Product Name	Catalog No.	Size
HighGene Transfection Reagent	RM09014	1mL
Instruction	-	1

1. Introduction of HighGene

HighGene transfection reagent is a new and highly effective cationic polymer. It interacts with nucleic acids (including plasmids, siRNA, and oligonucleotides) to form a compound that transports nucleic acids into eukaryotic cells and is suitable for the transfection of most eukaryotic cells.

2. Advantages of HighGene

（1）High transfection efficiency.

ABclonal selected common cell lines, such as 293T, HCT116 and C6, using HighGene transfection reagent and conventional market transfection reagent to perform cell transfection respectively. The experimental results are shown in Figure 1.

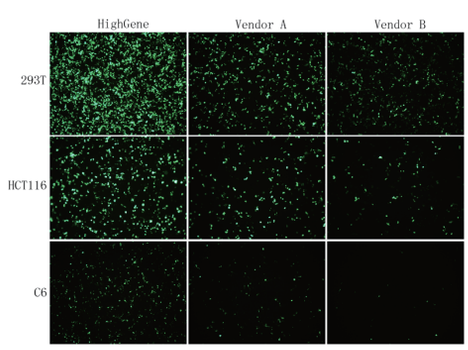

Figure 1: inoculated 293T, HCT116 and C6 cells in the 6-hole cell plate with a cell density of about 80%. Used HighGene transfection reagent and two similar products on the market for cell transfection respectively. The amount of GFP eukaryotic expression plasmids was 2μg. Observed the cells under a fluorescence microscope and took photo for the cells 48 hours after transfection.

（2）Stable batch and good repeatability.

ABclonal selected 293T and HeLa cell lines, using HighGene transfection reagent to perform cell transfection. The experimental results are shown in Figure 2.

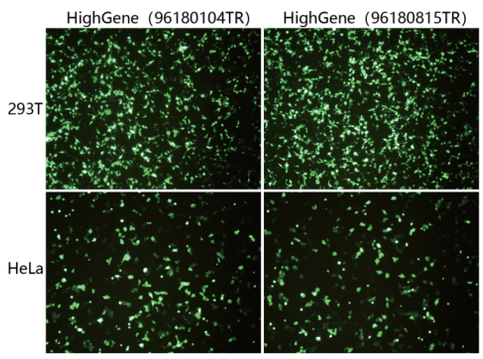

Figure 2: inoculated 293T and HeLa cells in the 6-hole cell plate with a cell density of about 70%. The amount of GFP eukaryotic expression plasmids was 2μg and that of the two different batches of HighGene transfection reagent was 4μL in cell transfection. Observed the cells under a fluorescence microscope and took photo for the cells 24 hours after transfection.

（3）Applicable to adherent cell and suspension cell.

HighGene transfection reagent has a good transfection effect for adherent cell and suspension cell. ABclonal selected HEK293F and Sf9 suspension cells to perform an experiment. The experimental results are shown in Figure 3.

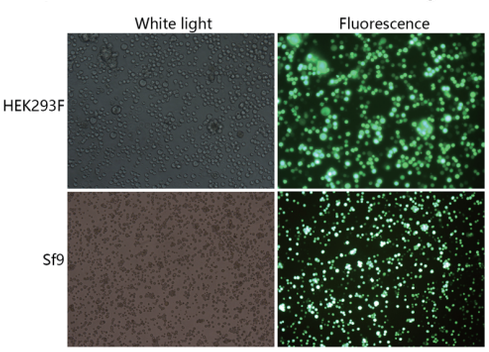

Figure 3: inoculated 30mL of HEK293F with a density of 1 x10^6 cells/mL or Sf9 suspension cell into a 125mL shake flask. The amount of GFP eukaryotic expression plasmids was 30μg and that of HighGene transfection reagent was 60μL in cell transfection. Observed the cells under a fluorescence microscope and took photo for the

cells 48 hours after transfection.

（4） No obvious cytotoxicity.

ABclonal selected 293T, HeLa, and HCT116 cell lines for cell transfection without changing cell culture medium and cultured continuously for 1-2 days before testing cell activity. The experimental results were shown in Figure 4.

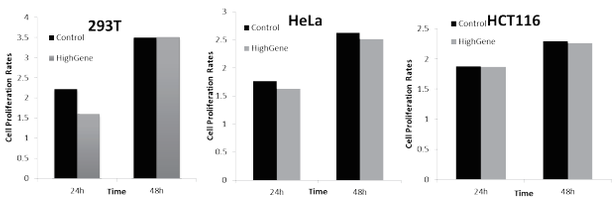

Figure 4: inoculated HEK293T, HeLa and HCT116 cells in 96-hole cell plate, with 5,000 cells in each hole and cell density of about 50%, and prepared 6 repeated holes. Treated cells with HighGene transfection reagent of 2μL/mL for 24h and 48h respectively and then used CCK8 reagent to test the cell viability.

（5） Applicable for siRNA cell transfection.

ABclonal selected 293T and Hela cell lines to perform siRNA transfection test, and respectively transfected siRNA and YAP1 siRNA with Cy3 fluorescence labeling. The experimental results were shown in Figure 5.

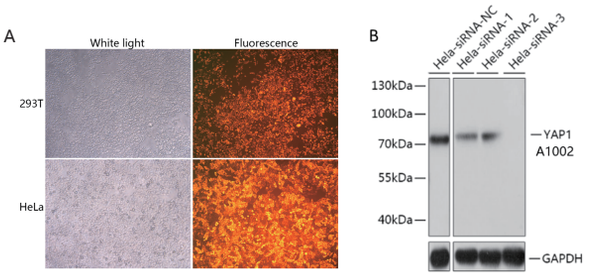

Figure 5: inoculated 293T and HeLa cells in the 6-hole cell plate with a cell density of about 80%. The amount of Cy3 siRNA was 100pmol and that of HighGene transfection reagent was 5μL, or that of YAP1 siRNA was 200pmol and that of HighGene transfection reagent was 10μL in cell transfection. Observed the cells under a fluorescence microscope and took photo (A) for the cells 24 hours after transfection or prepare cell sample to perform WB experiment (B) . (Remark: YAP1 antibody used in WB experiment is from ABclonal, with a catalog No. A1002.)

（6） Applicable to plasmid co-transfection

ABclonal selected 293T cell line to perform plasmid co-transfection test, and transfected green fluorescent protein and red fluorescent protein. The experimental results were shown in Figure 6.

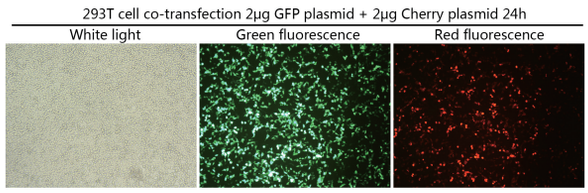

Figure 6: inoculated 293T cells in the 6-hole cell plate with a cell density of 80%. The amount of GFP eukaryotic expression plasmid was 2μg, that of Cherry eukaryotic expression plasmid was 2μg and that of HighGene transfection reagent was 8μL in cell transfection. Observed the cells under a fluorescence microscope and took photo for the cells 24 hours after transfection.

（7） Applicable to lentivirus packaging and infection experiment.

ABclonal selected 293T cell line to perform lentivirus packaging and then used lentivirus liquid to infect A549 cell line. The experimental results were shown in Figure 7.

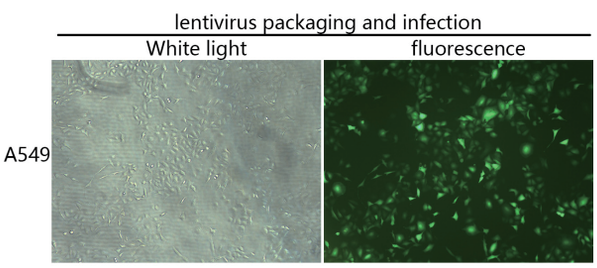

Figure 7: inoculated 293T cells in a 10cm culture dish with a cell density of 80%. Performed cell transfection for lentivirus packaging plasmids and external GFP expression plasmids, and got lentivirus liquid after 48 hours. Took 200uL lentivirus liquid to infect the A549 cells in the 6-hole cell plate, observed the cells under a fluorescence microscope and took photo for the cells 48 hours after transfection.

3. Storage Conditions

It shall be stored at 2-8℃ and valid for two years.

4. HighGene Instruction

4.1 Transfection of Adherent Cell (293T Cell as An Example)

(1) On the first day, 293T cells shall be inoculated into the 6-hole cell plates, and the cell density should be controlled at 70%-90%;

(Note: according to the experimental requirements, different cell culture devices can

(2) On the second day, add 2μg plasmid into centrifugal tube containing 100μL serum-free DMEM basic medium, and label it as solution A; and add 4μL HighGene transfection reagent to another centrifugal tube containing 100μL serum-free DMEM basic medium, label it as solution B.

(Note: such basic medium as MEM, 1640 and F12 can be used for the solvent of HighGene transfection reagent, and the amount of plasmids and dosage of HighGene transfection reagent required by different cell culture devices are indicated in Attached Table 2.)

(3) Slowly add 100μL solution B containing HighGene transfection reagent into 100μL solution A containing plasmids, blow and beat slowly with a pipette for several times, and set aside for 15 - 20 minutes.

(4) Evenly add 200μL HighGene transfection reagent / plasmids compound into the 6-hole cell plate and slightly shake the cell plate to distribute them evenly;
(Note: the 6-hole cell plate contains the complete medium, so slightly shake the cell plate to prevent cells from falling down and floating!)

(5) After 4-6h of cell transfection, replace with half of the fresh and complete medium;
(Note: during such replacement, absorb and discard half of the original complete medium and add half of the fresh complete medium.)

(6) After 24-48h of cell transfection, appropriate methods such as RT-PCR, Western, ELISA, reporter gene and others can be used for testing, or appropriate screening drugs (G418 or Puromycin) could be added to obtain stable cell lines.

4.2 Transfection of Suspension Cell (Setting HEK293F Cell as An Example)

(1) On the first day, inoculate 30mL HEK293F suspension cell with a cell density of 1×10^6 cells/mL into the 125mL shake flask;

(2) On the second day, take 30μg plasmids and add it into the centrifugal tube containing 1.5μL serum-free DMEM basic medium, and label it as solution A; Add 60μL HighGene transfection reagent to another centrifugal tube containing 1.5mL serum-free DMEM basic medium, label it as solution B;

(3) Slowly add 1.5mL solution B containing HighGene transfection reagent into 1.5mL solution A containing plasmids, blow and beat slowly with a pipette for several times, and set aside for 15 - 20 minutes.

(4) Evenly add 3mLHighGene transfection reagent / plasmids compound into the 125mL shake flask containing 30mL HEK293F suspension cell and slightly shake the shake flask to mix them evenly;

(5) After 5 days of cell transfection, collect cells or cell culture supernatant according to the protein expression (intracellular expression or secretory expression) for subsequent protein purification.

4.3 Transfection of siRNA Cell (Setting HeLa Cell as An Example)

(1) On the first day, Hela cells shall be inoculated into the 6-hole cell plates, and the cell density should be controlled at 70%-90%;

(2) On the second day, add 100pmol siRNA into centrifugal tube containing 100μL serum-free DMEM basic medium, and label it as solution A; and add 5μL HighGene transfection reagent to another centrifugal tube containing 100μL serum-free DMEM basic medium, label it as solution B. **(Note: the amount of siRNA and dosage of HighGene transfection reagent required by different cell culture devices are indicated in Attached Table 3.)**

(3) Slowly add 100μL solution B containing HighGene transfection reagent into 100μL solution A containing plasmids, blow and beat slowly with a pipette for several times, and set aside for 15 - 20 minutes.

(4) Evenly add 200μL HighGene transfection reagent / plasmids compound into the 6-hole cell plate and slightly shake the cell plate to distribute them evenly;

(Note: the 6-hole cell plate contains the complete medium, so slightly shake the cell plate to prevent cells from falling down and floating!)

(5) After 4-6h of cell transfection, replace with half of the fresh and complete medium;

(Note: during such replacement, absorb and discard half of the original complete medium and add half of the fresh complete medium.)

(6) After 24-48h of cell transfection, appropriate methods such as RT-PCR, Western, ELISA, reporter gene and others can be used for testing.

4.4 Lentivirus Packaging and Infection (A549 Cell as An Example)

(1) On the first day, 293T cells shall be inoculated into the 10cm culture dish, and the cell density should be controlled at 70%-90%;

(2) On the second day, respectively take and add lentivirus packaging plasmid pMD2G 4μg, lentivirus packaging plasmid pSPAX2 3μg, 6μg expression plasmids into centrifugal tube containing 500μL serum-free DMEM basic medium, and label it as solution A; and add 26μL HighGene transfection reagent to another centrifugal tube containing 500μL serum-free DMEM basic medium, label it as solution B;

(3) Slowly add 500μL solution B containing HighGene transfection reagent into 500μL solution A containing plasmids, blow and beat slowly with a pipette for several times, and set aside for 15 - 20 minutes.

(4) Evenly add 1mL HighGene transfection reagent / plasmids compound into the 10cm culture dish and slightly shake the culture dish to distribute them evenly;

(5) After 4-6h of cell transfection, replace with half of the fresh and complete medium;

(6) After 48h of cell transfection, collect the supernatant of cell culture by conventional centrifugation and filter it with 0.45um filter. Sub-package the supernatant every 200uL and store at -80℃ for later use.

(7) Inoculate A549 to be infected, and the cell density should be controlled at 70%-90%;

(8) After cells stick to the wall, evenly add 200μL lentivirus liquid into the 6-hole cell plate and slightly shake to distribute them evenly;

(9) After 18h of lentivirus infection, replace with the fresh and complete medium;

(10) After 48h of lentivirus infection, appropriate methods such as RT-PCR, Western, ELISA, reporter gene and others can be used for testing, or appropriate screening drugs (G418 or Puromycin) could be added to obtain stable expression cell lines.

5. Notes

(1) Before transfection, cells should be in a good growth state, with logarithmic growth period as the best. Transfection is recommended within 12-24 hours after cell passage and when cell density is 70-90%;

(2) The use of high quality plasmids is good for obtaining high transfection efficiency. It is recommended to use endotoxin-free plasmid extraction kit to extract plasmids. The A260/A280 ratio is 1.8 ~ 2.0, and the plasmid concentration is above 300ng/μL;

(3) When the transfection reagent and plasmid complex are prepared, the basic medium without serum and antibiotics shall be used as the solvent. The complete medium in the cell culture hole will not affect the cell transfection efficiency;

(4) In cell transfection experiments, the ratio of plasmid to transfection reagent can be appropriately adjusted according to cell transfection efficiency. The ratio of general plasmid amount (μg) to HighGene transfection reagent amount (μL) is between 1:1 - 1:4, and **the recommended ratio is 1:2;**

As for the virulence sensitive cells, it is recommended to replace with half of the fresh and complete medium after 4-6h of cell transfection;

(5) If the transfection reagent shows precipitation, gently shake the flask body until the precipitation dissolves; and

This product is only used for scientific research, not for clinical diagnosis and treatment.

Attached Table 1

Recommended amount and volume of cells inoculated with commonly used cell culture devices on the day before transfection

Cell Culture Device	Amount of Cells Loculated	Volume of Cell Medium
96-hole cell plate	(1-3)x10^4	0.1-0.2mL
24-hole cell plate	(1-3)x10^5	0.5-1mL
12-hole cell plate	(2-4)x10^5	1-2mL
6-hole cell plate	(3-5)x10^5	2-3mL
60mm culture dish	(5-10)x10^5	3-5mL
100mm culture dish	(1-3)x10^6	8-10mL
125ml shake flask	(1.5-2.5)x10^7	30-35mL
500ml shake flask	(6-10)x10^7	120-140mL
1000ml shake flask	(1.2-2)x10^8	240-280mL

Attached Table 2

Recommended dosage for cell transfection in common cell culture devices (plasma granulocytes transfection)

Cell Culture Device	Plasmid (μg)	HighGene Transfection Reagent (μl)	Compound Solution (μl)
96 hole cell plate	0.2	0.4	10
24 hole cell plate	1	2	50
12 hole cell plate	2	4	100
6 hole cell plate	2-4	4-8	200
60mm culture dish	3-5	6-10	400
100mm culture dish	5-10	10-20	1000
125mL shake flask	30-35	60-70	3000
500mL shake flask	120-140	240-280	12000
1000mL shake flask	240-280	480-560	24000

Attached Table 3

Recommended dosage for cell transfection in common cell culture devices (siRNA cell transfection)

Cell Culture Device	siRNA (pmol)	Transfection Reagent (μl)	Compound Solution (μl)
96 hole cell plate	4	0.2	10
24 hole cell plate	20	1	50
12 hole cell plate	40	2	100
6 hole cell plate	100	5	200
60mm culture dish	200	10	400
100mm culture dish	600	30	1000