PGL3 series Plasmid transfection

1. Plate the cells one day prior to transfection and switch to antibiotic-free 10% FBS DMEM medium and place 1X105 per well in 24-well plates. According to PGL3-Basic, PGL3-Hulc, PGL3-hTERT, PGL3-Control four groups, each group has four duplicate wells, totaling 16 wells.

2. Dilute Lipofectamine 3000 reagent in opti-MEM medium, 1.5 μL Lipofectamine 3000 per tube. Therefore, the medium 25*38 = 950 μl and Lipofectamine 3000 1.5*38 = 57 μl of a total of 1007 μl of the solution were uniformly mixed.

3. Dilute P3000 reagent in opti-MEM medium, prepare according to 2μl/μg plasmid, and mix each group with pRL-tk plasmid in a 10:1 relationship, ie 450ng+45ng/well, to ensure the total amount is 500 ng / hole. Therefore, there are the following systems:

(1) PGL3-Basic: medium 25*8=200 μl Basic plasmid 17 μl pRL-tk plasmid 1 μl P3000 1*8=8 μl

(2) For PGL3-Hulc: medium 25*8=200 μl hulc plasmid 11 μl pRL-tk plasmid 1 μl P3000 1*8=8 μl

(3) For PGL3-hTERT: medium 25*8=200 μl hTERT plasmid 12 μl pRL-tk plasmid 1 μl P3000 1*8=8 μl

(4) For PGL3-control: medium 25*8=200 μl Control plasmid 5.7 μl pRL-tk plasmid 1 μl P3000 1*8=8 μl

4. Mix the solutions in step 2 and step 3 uniformly (200 μl plus 200 μl) for 15 minutes, then add 50 μl per well to the 950 μl opti-MEM medium/ The wells were incubated in a 24-well plate in the 37 ° C ,5% CO2 incubator.

5. After 6H, switch to 10% DMEM medium and continue to culture, and test after 36h.