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RetroMax Transfection Buffer Kit

Catalog No. NBP2-29544

Contents: 1 bottle of 2X transfection buffer (10 mL) and 1 bottle of 2M $CaCl_2$ (2 mL)

BACKGROUND:

This is a part of the RetroMax expression system (Cat# NBP2-29499) and has been designed to maximize transfection efficiency of the RetroMax plasmids into mammalian cells. The following protocol may be followed to transfect pCL vectors into mammalian cell lines.

1. A day before transfection, seed 1 x 10^6 293 cells in 6 cm tissue culture plates. This should yield a cell density of about 30% confluence on the day of the experiment. You may also use 10-cm plates, and seed 2 x 10^6 cells if you would like to scale up.
2. Incubate overnight at 37°C in DMEM supplemented with 10% fetal bovine serum, pen-strep.
3. Add 0.25 mL (0.5 mL for 10 cm plates) of 2X transfection buffer (previously tested for optimum transfection efficiency) to the required number of sterile 15-mL polypropylene tubes (Falcon 2059).
4. Add the following to each required 1.5 mL sterile Eppendorf tube:
   - 30 µL of 2M $CaCl_2$ (included in the kit) and 220 µL sterile distilled water (60 µL of 2M $CaCl_2$ and 440 µL of distilled water for 10 cm plate).
   - 10 µg pCL-Eco, pCL-Ampho, or pCL-10A1.
   - 10 µg Retroviral Vector containing your gene of interest. If you are transfecting 10-cm plates (in 10 mL of medium) use 15-20 µg of each plasmid (30-40 µg total DNA) in 0.5 mL 0.25 M $CaCl_2$. Mix by vortexing.
5. Add the DNA/$CaCl_2$ mix dropwise to the transfection buffer tubes while lightly vortexing. Incubate at room temperature for 20 min (this step may be omitted). Add the DNA/$CaPO_4$ mix dropwise to 293 cells seeded the day before on 6-cm TC plates. Place in humidified CO_2 incubator for 3-4 hrs. (Longer times may result in cells coming off the plates. However, we have left the cells in the transfection medium overnight without loss of cells).
6. Carefully aspirate medium. Add 2 mL of warm PBS-15% Glycerol (no serum) for 2 min. *This step is optional*. In some cases, it may increase the transfection efficiency by 2-fold.
7. Aspirate glycerol shock medium. Carefully add 4 ml DMEM-10% fetal calf serum along the side of the dish. (10 mL for 10-cm plates). Incubate for 12-18 hrs.
8. Next day, aspirate medium and add fresh medium in the morning. This step helps dilute out a cytostatic factor that is produced by the transfected cells (both 293 and COS produce it; untransfected cells do not).

STORAGE:

For long-term storage, store at 4°C.

REFERENCES:

1. Naviaux, RK, Costanzi, E, Haas, M and Verma, I. The pCL vector system: Rapid production of helper-free, high titer, recombinant retroviruses. *J. Virol* 70: 5701-5705 (1996).