GeticoFect HQ Transfection Reagent Instruction Manual for Transfection in Multiple Cell Types

I. General Preparation

1. Reagents and Materials

- **GeticoFect HQ Transfection Reagent** (with HQ-ER Enhancer)
- Serum-free medium (e.g., Opti-MEM or DMEM/F12)
- Endotoxin-free plasmid DNA (concentration ≥0.5 μg/μL, OD260/280 = 1.8-2.0)
- Complete medium (adjusted to cell type, supplemented with 10% FBS and antibiotics)
- 12-well/24-well cell culture plates, sterile microcentrifuge tubes, pipettes

2. Cell Culture Requirements

- Cells must be in logarithmic growth phase with viability >90%.
- Seed cells 1 day before transfection at densities specified for each cell line.
- Avoid using cells beyond recommended passage numbers (e.g., primary cells <5 passages).

II. Transfection Procedures for Each Cell Line

1. THP-1 Cells (Human Monocytic Leukemia Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 10% FBS + 1% P/S
- Seeding density: 1×10^6 cells/well (12-well plate), 50%-60% confluency at transfection

Transfection Steps:

1. **Complex Preparation** (per well):
    - **Solution A**: 2 μg plasmid DNA + 100 μL Opti-MEM
    - **Solution B**: 4 μL GeticoFect HQ + 2 μL HQ-ER + 100 μL Opti-MEM
    - Incubate solutions separately for 5 min, then combine and incubate for 20 min at RT.
2. **Transfection**:
    - Replace cell medium with 1 mL fresh complete medium.
    - Add complexes dropwise and gently swirl the plate.
    - Incubate at 37°C for 4-6 hours, then replace with fresh medium.

Analysis Time: Assay gene expression 48-72 hours post-transfection.

2. MCF-7 Cells (Human Breast Adenocarcinoma Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70%-80% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.8 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Mix and incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh complete medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

3. RAW264.7 Cells (Mouse Macrophages)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 3×10^5 cells/well (12-well plate), 50% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1.5 μg plasmid DNA + 100 μL Opti-MEM
- **Solution B**: 3 μL GeticoFect HQ + 1.5 μL HQ-ER + 100 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 1 mL fresh complete medium.
- Add complexes and incubate for 4 hours, then replace medium.

Analysis Time: Assay 24-48 hours post-transfection.

4. Primary Mouse Neural Progenitor Cells

Pre-transfection Setup:

- Medium: Neurobasal Medium + B27 + GlutaMAX
- Seeding density: 2×10^5 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.5 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 1.5 μL GeticoFect HQ + 0.5 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 4 hours, then replace medium.

Analysis Time: Assay 72 hours post-transfection.

5. PC12 Cells (Rat Pheochromocytoma Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 10% FBS + 5% HS + 1% P/S
- Seeding density: 3×10^4 cells/well (24-well plate), 50%-60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.8 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

6. NHFF Cells (Normal Human Foreskin Fibroblasts)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 8×10^4 cells/well (12-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 2 μg plasmid DNA + 100 μL Opti-MEM
- **Solution B**: 4 μL GeticoFect HQ + 2 μL HQ-ER + 100 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 1 mL fresh medium.
- Add complexes and incubate for 4 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

7. NIH/3T3 Cells (Mouse Embryonic Fibroblasts)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.8 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

8. SKBR-3 Cells (Human Breast Adenocarcinoma Cells)

Pre-transfection Setup:

- Medium: McCoy's 5A + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70%-80% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

9. LNCaP Cells (Human Prostate Carcinoma Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 µg plasmid DNA + 50 µL Opti-MEM
- **Solution B**: 2.5 µL GeticoFect HQ + 1 µL HQ-ER + 50 µL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

10. K562 Cells (Human Chronic Myelogenous Leukemia Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 10% FBS + 1% P/S
- Seeding density: 1×10^6 cells/mL (24-well plate, 1 mL/well)

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 2 μg plasmid DNA + 100 μL Opti-MEM
- **Solution B**: 5 μL GeticoFect HQ + 2 μL HQ-ER + 100 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Centrifuge cells (1200 rpm, 5 min), resuspend in 1 mL fresh medium.
- Add complexes, mix gently.
- Incubate for 24 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

11. Jurkat Cells (Human T-Cell Leukemia Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 10% FBS + 1% P/S
- Seeding density: 1×10^6 cells/mL (24-well plate, 1 mL/well)

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 2 μg plasmid DNA + 100 μL Opti-MEM
- **Solution B**: 5 μL GeticoFect HQ + 2 μL HQ-ER + 100 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Centrifuge cells (1200 rpm, 5 min), resuspend in 1 mL fresh medium.
- Add complexes, mix gently.
- Incubate for 24 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

12. IMR-90 Cells (Human Lung Fibroblasts)

Pre-transfection Setup:

- Medium: MEM + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.8 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

13. HUVEC Cells (Human Umbilical Vein Endothelial Cells)

Pre-transfection Setup:

- Medium: EGM-2 Endothelial Cell Medium
- Seeding density: 5×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 0.8 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 4 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

14. HT-1080 Cells (Human Fibrosarcoma Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2.5 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

15. HL-60 Cells (Human Promyelocytic Leukemia Cells)

Pre-transfection Setup:

- Medium: RPMI 1640 + 20% FBS + 1% P/S
- Seeding density: 1×10^6 cells/mL (24-well plate, 1 mL/well)

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 2 μg plasmid DNA + 100 μL Opti-MEM
- **Solution B**: 5 μL GeticoFect HQ + 2 μL HQ-ER + 100 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Centrifuge cells (1200 rpm, 5 min), resuspend in 1 mL fresh medium.
- Add complexes, mix gently.
- Incubate for 24 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

16. HepG2 Cells (Human Hepatocellular Carcinoma Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2.5 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

17. HeLa S3 Cells (Human Cervical Carcinoma Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

18. HCT-116 Cells (Human Colorectal Carcinoma Cells)

Pre-transfection Setup:

- Medium: McCoy's 5A + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 60%-70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2.5 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

19. GripTite 293 MSR Cells (Human Embryonic Kidney Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 70% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 µg plasmid DNA + 50 µL Opti-MEM
- **Solution B**: 2 µL GeticoFect HQ + 1 µL HQ-ER + 50 µL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

20. COS-7 Cells (African Green Monkey Kidney Cells)

Pre-transfection Setup:

- Medium: DMEM + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2.5 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

21. CHO-K1 Cells (Chinese Hamster Ovary Cells)

Pre-transfection Setup:

- Medium: F-12K + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 µg plasmid DNA + 50 µL Opti-MEM
- **Solution B**: 2.5 µL GeticoFect HQ + 1 µL HQ-ER + 50 µL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48-72 hours post-transfection.

22. Caco-2 Cells (Human Colorectal Adenocarcinoma Cells)

Pre-transfection Setup:

- Medium: DMEM + 20% FBS + 1% P/S
- Seeding density: 5×10^4 cells/well (24-well plate), 50%-60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 3 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 72 hours post-transfection.

23. C6 Cells (Rat Glioma Cells)

Pre-transfection Setup:

- Medium: F-12K + 10% FBS + 1% P/S
- Seeding density: 4×10^4 cells/well (24-well plate), 60% confluency

Transfection Steps:

Complex Preparation (per well):

- **Solution A**: 1 μg plasmid DNA + 50 μL Opti-MEM
- **Solution B**: 2.5 μL GeticoFect HQ + 1 μL HQ-ER + 50 μL Opti-MEM
- Incubate for 20 min.

Transfection:

- Replace medium with 0.5 mL fresh medium.
- Add complexes and incubate for 6 hours, then replace medium.

Analysis Time: Assay 48 hours post-transfection.

III. Optimization Tips and Precautions

DNA Quality Control:

- Use endotoxin-free plasmid DNA (e.g., purified with EndoFree Maxi Kit).
- Concentration ≥0.5 μg/μL with OD260/280 ratio between 1.8-2.0.

Reagent Handling:

- Equilibrate GeticoFect HQ and HQ-ER to room temperature before use.
- Avoid repeated freeze-thaw cycles; aliquot and store at -20°C.
- Mix gently by inversion; do not vortex vigorously.

Cell Health:

- Cell confluency significantly impacts transfection efficiency; seed at recommended densities.
- Passage limits: Primary cells <5 passages, cell lines <20 passages.

Toxicity Management:

- If cytotoxicity occurs, try:
    - Reducing transfection reagent volume.
    - Shortening complex incubation time (e.g., 15 min).
    - Increasing media change frequency (first change 4 hours post-transfection).

Transfection Optimization:

- Perform dose-response experiments (e.g., DNA:reagent ratio from 1:1 to 1:5).
- For hard-to-transfect cells (e.g., primary cells):
    - Increase HQ-ER enhancer ratio (e.g., DNA:HQ-ER = 1:2).
    - Extend transfection time (e.g., 8-12 hours).

Controls:

- Positive control: Transfect EGFP-expressing plasmid to assess transfection efficiency.
- Negative control: Untransfected cells to measure background signal.

IV. Detection Methods

1. **Fluorescence Microscopy**: Directly visualize transfected cells 24-48 hours post-transfection if using a fluorescent reporter (e.g., GFP, RFP).

2. **Flow Cytometry**: Quantify transfection efficiency (recommended 48 hours post-transfection).
3. **Western Blot**: Detect protein expression levels (48-72 hours post-transfection).
4. **qRT-PCR**: Measure mRNA expression levels (24 hours post-transfection).