General protocol for transfection of stem cells, primary cells, and continuous cell lines with ATCC GeneX*Plus* Transfection Reagent

Guidelines for optimal plasmid DNA transfection

Transfection conditions should be optimized for each cell type to ensure maximal gene expression with minimal cellular toxicity. However, the general suggestions below have been demonstrated to yield high efficiency of transfections using GeneX*Plus* Transfection Reagent. **Table 1** presents recommended starting conditions based on culture vessel size and **Table 2** presents a detailed optimization schematic for transfection of a new cell type/line. In general, gene expression will increase, plateau and then decrease with increasing concentrations of the GeneX*Plus* Transfection Reagent. Gene expression also increases with increasing amounts of plasmid, then plateaus or even decreases**.** The amount DNA in the transfection complex directly affects toxicity and every cell type has an optimal amount of DNA. If toxicity is observed, reduce the amount of DNA and/or the amount of DNA /transfection complex added to cells. For optimal cell seeding density and ratio of GeneX*Plus* to DNA in commonly used cell types/lines, please refer an ATCC's cell-specific transfection protocol.

1. **Cell seeding and Cell density at transfection.** Cells should be plated 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density (generally 70–90% confluence) at the time of transfection.

2. **Cell passage number.** It is critical to transfect primary cells at early passages.

3. **DNA expression vector selection.** Promoter (CMV vs. EF1α), type of GFP (Enhanced GFP vs. Emerald GFP) and size of expression vector (5 kb vs. 8 kb) dramatically affect transgene expression in particular cell types.

4. **DNA Preparation**. Plasmid DNA must be sterile and free from phenol and other contaminants.

5. **Ratio of GeneX*Plus* Reagent to DNA.** Depending on the cell type, the optimal ratio of DNA (µg) to **GeneX*Plus*** Transfection Reagent (µL) varies from 1:1 to 1:3. A DNA (µg) to reagent (µL) ratio of 1:2 is recommended as a starting point.

6. **Complex formation conditions.** Prepare GeneX*Plus* Transfection Reagent and DNA complexes in serum-free growth medium (*e.g.* Opti-MEM Medium).

7. **Presence of antibiotics and other known inhibitors:** Antibiotics can inhibit transfection complex formation and therefore should be excluded from the complex formation step. However, GeneX*Plus* has been optimized for intracellular delivery of nuclei acids into cultured mammalian cells in the presence of serum. Culture medium containing polyanions such as heparin, heparin sulfate or dextran sulfate can also inhibit transfection. Medium containing these chemicals should not be used for transfection; however, the medium can be replaced with medium containing polyanions 24 hours after transfection.

8. **Post-transfection incubation time.** The optimal incubation time is generally 24–72 hours post transfection, but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time.

Transient plasmid DNA transfection protocol per well of a 12-well plate

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Or contact your local distributor.

***Note*:** Adjust volumes for GeneX*Plus* Transfection Reagent, DNA and complete growth medium based on the surface area of the cell culture vessel as described in **Table 1**.

Cell Seeding

Note: For higher transfection efficiency, it is recommended that the cells are > 85% viable at the time of transfection and are in early passages.

1. Approximately 18–24 hours before transfection, plate cells in 1 mL complete growth medium per well in a 12-well plate. Cells should be 70-90% confluent at the time of transfection.

   For adherent cells: Plate cells at a density of 1-4 × 10^5 cells/well.

   For suspension cells: Plate cells at a density of 6-8 × 10^5 cells/mL.
2. Incubate cell cultures overnight.
3. Replace medium with 1 mL of fresh complete growth medium except polyanions (*e.g.* heparin sulfate) prior to transfection.

Preparation of Transfection Reagent: DNA complex (prepare immediately before transfection)

1. Warm GeneX*Plus*™ Transfection Reagent to room temperature. Vortex gently before using.
2. Place 100 µL of serum-free medium (*e.g.* Opti-MEM) in a sterile tube.
3. Add 1 µg (1 µL of a 1 µg/µL stock) plasmid DNA to the medium in the tube. Mix completely by gently pipetting up and down.
4. Add 2 µL GeneX*Plus*™ Transfection Reagent to the diluted DNA mixture. Mix gently by tapping or briefly vortexing.
5. Centrifuge briefly to collect reaction mixture in bottom of the tube.
6. Incubate at room temperature for 20 minutes to allow sufficient time for complexes to form.

Addition of Complexes to Cells

1. Add the transfection complex drop-wise, to the 12-well plates containing cells in complete growth medium. Swirl plate gently after each addition.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the GeneX*Plus*™ Transfection Reagent: DNA complexes.
3. Incubate the cells overnight.
4. Change the complete growth medium with fresh medium next day and once every day thereafter.
5. Assess transgene expression 24 to 72 hours post-transfection.

For generating stable cell transfectants: Passage cells 24 to 48 hours post-transfection in complete growth medium containing appropriate selection antibiotics, such as G418 or Hygromycin B. Maintain selection for 1 to 2 weeks to allow for selection of cells that have undergone stable integration of DNA.

Please visit www.atcc.org/transfection for more information regarding lipid based transfection reagents, cell-specific protocols, as well as current promotions and free sample availability.

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Table 1. Recommended starting conditions for transfections with GeneX*Plus* Transfection Reagent

Culture vessel	96-well plate	24-well plate	12-well plate	6-well plate	6-cm dish	10-cm dish
Surface Area (cm^2)	0.35	1.9	3.8	9.6	21.5	59
Complete Growth Medium (mL)	0.1	0.5	1	2	5	10
Diluent (serum-free medium) (µL)	20	50	100	200	500	1000
Amount of DNA (µg)	0.1	0.5	1	2	5	10
GeneX*Plus* Transfection Reagent (µL)	0.2	1	2	4	10	20

Table 2. Transfection optimization setup in a 12-well plate

		1:1 ratio of DNA vs. Lipid	1:2 ratio of DNA vs. Lipid	1:3 ratio of DNA vs. Lipid	Neg. control
		1	2	3	4
0.5 µg DNA/well	A	0.5 µg DNA + 0.5 µL GeneX*Plus*	0.5 µg DNA in 1.0 µL GeneX*Plus*	0.5 µg DNA + 1.5 µL GeneX*Plus*	No transfection control
1.0 µg DNA/well	B	1.0 µg DNA + 1.0 µL GeneX*Plus*	1.0 µg DNA + 2.0 µL GeneX*Plus*	1.0 µg DNA + 3.0 µL GeneX*Plus*	DNA only control
2.0 µg DNA/well	C	2.0 µg DNA + 2.0 µL GeneX*Plus*	2.0 µg DNA + 4.0 µL GeneX*Plus*	2.0 µg DNA + 6.0 µL GeneX*Plus*	lipid only control

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