GeneX*Plus* Transfection of Plasmid DNA into BJ-5ta Cells

BJ-5ta (ATCC® No. CRL-4001™) are normal human foreskin fibroblast cells immortalized through the forced expressed of the hTERT component of Telomerase. ATCC has achieved transfection efficiencies of approximately **73%** using the protocol described below.

General Considerations for using the GeneX*Plus* transfection reagent:

- All steps should be performed in a biosafety cabinet using proper aseptic technique.
- **Cell conditions.** Cells should be passaged at least once after thaw and the use of low-passage cells is recommended. Passage the cells 18-24 hours before transfection to ensure the cells are actively dividing and that they will be at the appropriate cell density at the time of transfection. Make sure that the cells are healthy and are ≥ 90% viable, prior to transfection.
- **Seeding density.** Cell density should be 70-80% confluent on the day of transfection. See specified seeding density in the individual protocols and in Table 1. *Note: Determine the optimal cell density for each cell type in order to maximize transfection efficiency*.
- **DNA purity.** Use highly purified plasmid preps that are free from phenol or other contaminants. Plasmid DNA preps that are endotoxin-free are desirable.
- **Presence of antibiotics and other inhibitors.** Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing serum and low levels of antibiotics if required.
- **Complex formation conditions.** Prepare GeneX*Plus* reagent and DNA complexes in serum-free growth medium. ATCC recommends using Opti-MEM I Reduced-Serum Medium to dilute the DNA before complex formation.

Materials required:

Material Required	Catalog No.
BJ-5ta cells	ATCC® CRL-4001™
DMEM	ATCC® 30-2002
Hygromycin B	Sigma-Aldrich® H3274
FBS	ATCC® 30-2020
GeneX*Plus*	ATCC® ACS-4004
Opti-MEM® I Reduced-Serum Media	Life Technologies™ 31985-062
M199 Media	Life Technologies™ 11150059
Plasmid DNA of interest (1µg/µL)	
Tissue culture plates and supplies	

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
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Fax: 703-365-2750
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Or contact your local distributor.

Protocol:

The following protocol describes how to transfect plasmid DNA into BJ-5ta cells using the GeneX*Plus* Reagent in a **12 well dish.** The reaction can be scaled up as needed. Please refer to Table 1 for recommended reaction conditions for other dish or plate sizes.

A. Preparation of the cells for transfection

The day before transfection:

1. Count and measure cells for density and viability.
2. Plate **7.5 x 10^4** cells per well in 1.0 mL of complete growth medium. Cell density should be **70- 80%** confluent on the day of transfection.
3. Incubate cells overnight at **37°C** with **5% CO_2**.

The day of transfection:

1. Remove old media.
2. Replace old media with 1 mL of medium containing a 4:1 mixture of DMEM:M199 and 20% FBS without antibiotics.

B. Preparation of the DNA:TransfeX transfection complexes

1. Warm GeneX*Plus*, plasmid DNA, and Opti-MEM I Reduced-Serum Medium to room temperature and vortex gently to mix.
2. Pipette 100 µL Opti-MEM I Reduced-Serum Medium into a sterile microcentrifuge tube.
3. Add 1.0 µL (1.0 µg/µL) plasmid DNA.
4. Mix thoroughly with gently pipetting.
5. Add 4.0 µL GeneX*Plus* Reagent to the diluted DNA mixture. *Note: Do not let the pipette tip or the reagent come into contact with the sides of the plastic tube.*
6. Mix GeneX*Plus* complexes thoroughly using either a vortex or by pipetting briefly.
7. Collect contents at bottom of the tube using a mini-centrifuge.
8. Incubate GeneX*Plus*:DNA complexes at room temperature for 15 minutes.

C. Addition of DNA:GeneX*Plus* transfection complexes to the cells

1. Distribute the complexes to the cells by adding the complexes drop-wise to different areas of the wells.
2. Gently rock the culture vessel back and forth and from side to side to evenly distribute the GeneX*Plus*:DNA complexes.

D. Post-Transfection Handling

1. Incubate for **24-72** hours. Replace transfection medium with fresh complete growth medium every 24 hours post transfection.
2. Wait for 18-24 hours post-transfection before assaying for transgene expression.

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Table 1: Recommended Reaction Conditions for different size culture vessels.

Culture Vessel	96 well plate	24 well plate	12 well plate	6 well plate	10 cm dish
Surface area	0.35 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2
Cell seeding	0.7 x 10^4 per well	3.8 x 10^4 per well	7.5 x 10^4 per well	19 x 10^4 per well	118 x 10^4 per well
Complete Growth Medium	0.1 mL	0.5 mL	1.0 mL	2.0 mL	10 mL
Opti-MEM I Reduced Serum Medium	20 µL	50 µL	100 µL	200 µL	1.0 mL
DNA (1 µg/µL stock)	0.125 µg	0.5 µg	1.0 µg	2.0 µg	12 µg
TransfeX Reagent	0.5 µL	2.0 µL	4..0 µL	8.0 µL	24 µL

Notes:

1. If excessive cytotoxicity occurs, we recommend culturing BJ-5ta cells in antibiotic-free media for 24h (or more) prior to, and following transfection.
2. Decreasing the ratio of DNA: GeneX*Plus* (e.g., 1:2 or 1:3) may save reagent with only minor (~10%) decreases in transgene expression.

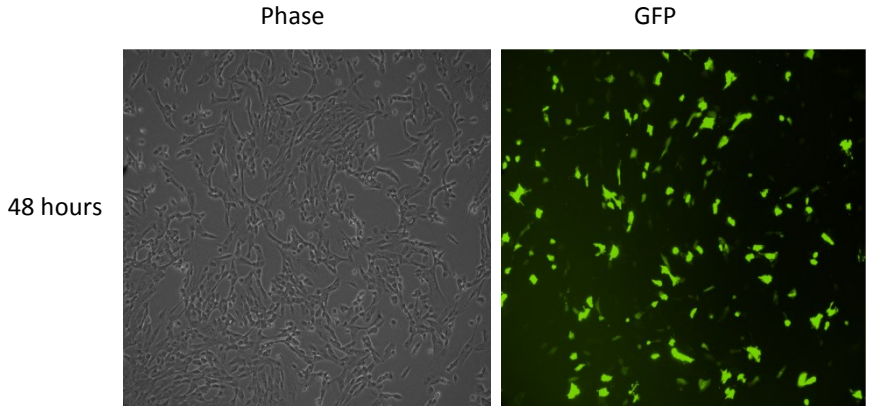

Figure 1. GFP expression in BJ-5ta (CRL-4001) transfected with 4 µL of GeneX*Plus* Transfection Reagent and 1 µg of an EF1α-eGFP vector per well of a 12 well plate day 2 post-transfection (10x)

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