



TransfeX™ Transfection of Plasmid DNA into Telomerase Immortalized Human Microvascular Endothelium (TIME) Cells

TIME cells (ATCC® Cat. No. CRL-4025™) are normal human microvascular endothelial cells immortalized through the forced expressed of the hTERT component of Telomerase. ATCC has achieved transfection efficiencies of approximately **50%** using the protocol described below.

General Considerations for using the TransfeX transfection reagent:

- All steps should be performed in a biosafety cabinet using proper aseptic technique.
- **Cell conditions.** Cells should be passage 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 $\geq 90\%$ 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 TransfeX 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. |
|--|------------------------------|
| TIME cells | ATCC® CRL-4025™ |
| Vascular Cell Basal Medium | ATCC® PCS-100-030 |
| Microvascular Endothelial Cell Growth Kit - VEGF | ATCC® PCS-110-041 |
| TransfeX™ | ATCC® ACS-4005 |
| Opti-MEM® I Reduced-Serum Media | Life Technologies™ 31985-062 |
| Plasmid DNA of interest (1µg/µL) | |
| Tissue culture plates and supplies | |

Protocol:

The following protocol describes how to transfect plasmid DNA into TIME cells using the TransfeX Reagent in a **single well of a 24-well plate**. The reaction may 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 **25,000** cells per well in complete growth medium (**Vascular Cell Basal Media supplemented with the Microvascular Endothelial Cell Growth Kit - VEGF**). Cell density should be **75%** confluent on the day of transfection.
3. Incubate cells overnight at **37°C** with **5% CO₂**.

The day of transfection:

1. Remove old media.
2. Replace old media with fresh complete growth media (excluding Heparin Sulfate) to a total volume of 0.5 mL/well.

B. Preparation of the DNA:TransfeX transfection complexes

1. Warm TransfeX, plasmid DNA, and Opti-MEM I Reduced-Serum Medium to room temperature and vortex gently to mix.
2. Pipette 50 μ L Opti-MEM I Reduced-Serum Medium into a sterile microcentrifuge tube.
3. Add **0.25 μ L** (1.0 μ g/ μ L) plasmid DNA.
4. Mix thoroughly with gently pipetting.
5. Add **0.75 μ L** TransfeX 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 TransfeX:DNA complexes thoroughly using either a vortex or by pipetting briefly.
7. Collect contents at bottom of the tube using a mini-centrifuge.
8. Incubate TransfeX:DNA complexes at room temperature for 15 minutes.

C. Addition of DNA:TransfeX transfection complexes to the cells

1. Distribute the complexes to the cells by adding **50 μ L** of 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 TransfeX:DNA complexes.

D. Post-Transfection Handling

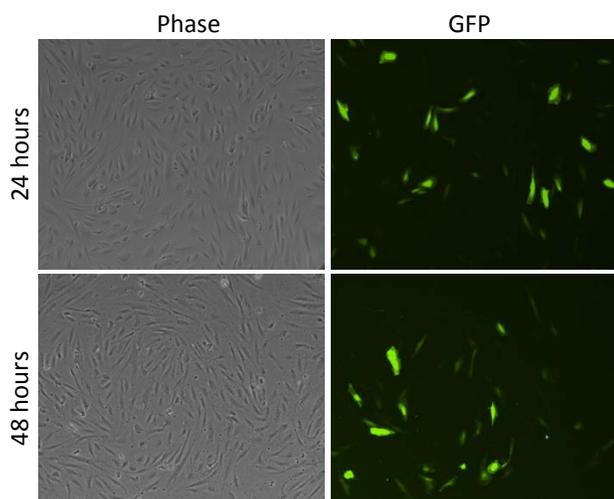
1. Incubate for **24-72** hours. Replace transfection medium with fresh complete growth medium every 24 hours post transfection.
2. Wait for 18-24 hours post-transfection before assaying for transgene expression.

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 | 4.8 x 10 ⁴ per well | 9.6 x 10 ⁴ per well | 20 x 10 ⁴ per well | 120 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) | 0.06 µg | 0.25 µg | 0.5 µg | 1.0 µg | 6.0 µg |
| TransfeX Reagent | 0.18 µL | 0.75 µL | 1.5 µL | 3.0 µL | 18 µL |

Notes:

1. If cell viability is too low post transfection make sure cell density is at 75% at the time of transfection, and that the correct ratio of TransfeX:DNA was used
2. Include a control condition consisting of an empty vector plasmid or a plasmid expressing GFP.



Transfection efficiency of TransfeX reagent on TIME cells. Cells were transfected with EF1α-eGFP empty vector at 0.25 µg DNA with 0.75 µL of reagent (1:3) in Opti-MEM I Reduced Serum Media.