THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

# TransfeX™ Reagent General Protocol

Transfection conditions should be optimized for each cell type to ensure maximal gene expression and minimal cellular toxicity. However, the general suggestions below have been demonstrated to yield high efficiency transfections using the TransfeX™ Transfection reagent. **Table 1** presents recommended starting conditions based on culture vessel size. For optimal cell seeding density and ratio of TransfeX to DNA for a particular cell type, please refer to the cell-specific transfection protocol.

**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 ≥ 90% viable, prior to transfection.
- **Seeding density.** Cell density should be 50-80% 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. Culture medium containing polyanions such as heparin, heparin sulfate or dextran sulfate can inhibit transfection. Medium containing these chemicals should not be used for transfection. However, the medium can be replaced with complete growth medium 24 hours after transfection.
- **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.
- **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.
- **RNA transfection.** TransfeX has been tested to deliver siRNA as well as mRNA, however we do not provide cell line specific protocols. In general, we recommend using a greater volume of reagent when forming complexes with RNA than with DNA for the same cell type.

**Materials required**:

| Material Required | Catalog No. |
|---|---|
| Cells of Interest growing in recommended media conditions | See cell-specific product sheets |
| TransfeX™ | ATCC® ACS-4005 |
| Opti-MEM® I Reduced-Serum Media | Life Technologies™ 31985-062 |
| Plasmid DNA of interest (1 µg/µL) | |
| Tissue culture plates and supplies | |

**American Type Culture Collection**
P.O. Box 1549
Manassas, VA 20108 USA
http://www.atcc.org

800-683-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

**Protocol:**

The following protocol describes a general protocol for the transient transfection of plasmid DNA into cells using the TransfeX Reagent in a **single well of a 12 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. *Note: For higher transfection efficiency, it is recommended that the cells are > 85% viable at the time of transfection and are in early passages.*
2. Plate cells in complete growth medium. Cell density should be such that the cells are **50-80%** confluent on the day of transfection. See cell line specific product sheets for suggested plating densities, but in general the following densities are appropriate:
    a. ***For adherent cells:*** Plate cells at a density of 1-4 × $10^5$ cells/well.
    b. ***For suspension cells:*** Plate cells at a density of 6-8 × $10^5$ cells/mL.
3. Incubate cells overnight at **37°C** with **5% $CO_2$**.

The day of transfection:

1. Remove old media.
2. Replace old media with fresh complete growth media (omit polyanions like heparin sulfate) to a total volume of 1.0 mL

**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 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 2.0 µL 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 transfection complexes to 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 TransfeX:DNA complexes.
3. Incubate for **24-72** hours. Replace transfection medium with fresh complete growth medium every 24 hours post transfection. *Note: Please see cell line specific protocols for specific post-transfection recommendations.*

**American Type Culture Collection**
P.O. Box 1549
Manassas, VA 20108 USA
http://www.atcc.org

800-683-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

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

| Culture Vessel | 24 well plate | 12 well plate | 6 well plate | 10 cm dish |
|---|---|---|---|---|
| Surface area | 1.9 $cm^2$ | 3.8 $cm^2$ | 9.6 $cm^2$ | 59 $cm^2$ |
| Complete Growth Medium | 0.5 mL | 1.0 mL | 2 mL | 10 mL |
| Opti-MEM I Reduced Serum Medium | 50 µL | 100 µL | 200 µL | 1 mL |
| DNA (1 µg/µL stock) | 0.5 µg | 1.0 µg | 2 µg | 10 µg |
| TransfeX Reagent | 1 µL | 2 µL | 4 µL | 20 µL |

**American Type Culture Collection**
P.O. Box 1549
Manassas, VA 20108 USA
http://www.atcc.org
800-683-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.