



# GeneXPlus Transfection Reagent

ACS-4004™

## Description

GeneXPlus Transfection Reagent is a broad-spectrum reagent that provides exceptional transfection of plasmid DNA into mammalian cells. This reagent affords high levels of gene expression in a variety of cell types and is suitable for both transient and stable transfection.

**Volume:** 1.0 mL

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## Storage Conditions

**Storage conditions:** -20°C or colder

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## Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

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## BSL 1

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understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Handling Procedures

### Guidelines for optimal plasmid DNA transfection

Reaction conditions should be optimized for each cell type to ensure successful transfections. However, the general suggestions below have been demonstrated to yield high efficiency transfections using GeneXPlus Transfection Reagent. **Table 1** presents recommended starting conditions based on culture vessel size.

1. **Cell seeding and Cell density at transfection.** Cells should be plated 18 to 24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density (generally 40–80% confluence) at the time of transfection.
2. **DNA Preparation.** Plasmid DNA must be sterile and free from phenol and other contaminants.
3. **Ratio of GeneXPlus Reagent to DNA.** Depending on the cell type, the optimal ratio of DNA ( $\mu\text{g}$ ) to GeneXPlus Transfection Reagent ( $\mu\text{L}$ ) varies from 1:1 to 1:4. A DNA ( $\mu\text{g}$ ) to reagent ( $\mu\text{L}$ ) ratio of 1:3 is recommended as a starting point.
4. **Complex formation conditions.** Prepare GeneXPlus Transfection Reagent and DNA complexes in serum-free growth medium.
5. **Presence of antibiotics and other known inhibitors.** Antibiotics can inhibit transfection complex formation and therefore should be excluded from the complex formation step. Culture medium containing polyanions such as

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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.

6. **Post-transfection incubation time.** The optimal incubation time is generally 24 to 72 hours post transfection, but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time. Some secreted proteins can be expressed for up to 7 days post transfection.
7. **Reaction size.** For protein expression, it is critical to have adequate shaking in the well. Experiments have shown that yields in a 6-well plate can be lower than those obtained in a 10 mL reaction size (or greater) in shaker flasks, but is still representative of the selected transfection conditions.

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

Culture vessel	96-well plate	24-well plate	12-well plate	6-well plate	10-cm dish	T75 flask	125-mL shaker flask
Surface area (cm <sup>2</sup> )	0.35	1.9	3.8	9.6	59	75	N/A
Complete Growth Medium (mL)	0.092	0.5	1	2.5	15.5	19.7	20
Diluent (serum-free medium) ( L)	9	50	100	250	1500	1900	2000
Amount of DNA ( g)	0.1	0.5	1	2.5	15	19	20

Transfection reagent ( L )	0.3	1.5	3	7.5	45	57	60
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### Transient plasmid DNA transfection protocol per well of a 6-well plate

**Note:** Adjust volumes for GeneXPlus 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 > 80% viable at the time of transfection.

1. Approximately 18–24 hours before transfection, plate cells in 2.5 mL complete growth medium per well in a 6-well plate. Cells should be 40–80% confluent prior to transfection.

**For adherent cells:** Plate cells at a density of  $2 \times 10^5$  to  $6 \times 10^5$  cells/well.

**For suspension cells:** Plate cells at a density of  $6 \times 10^5$  to  $8 \times 10^5$  cells/mL.

2. Incubate cell cultures overnight.

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

1. Warm GeneXPlus Transfection Reagent to room temperature. Vortex gently before using.
2. Place 250  $\mu$ L of serum-free complete growth medium in a sterile tube.
3. Add 2.5  $\mu$ g (2.5  $\mu$ L of a 1  $\mu$ g/ $\mu$ L stock) plasmid DNA to the medium in the tube. Mix completely by gently pipetting up and down.
4. Add 7.5  $\mu$ L GeneXPlus Transfection Reagent to the diluted DNA mixture. Do not allow reagent to come in contact with the sides or bottom of the tube. Mix completely by gently pipetting.
5. Centrifuge briefly to collect reaction mixture in bottom of the tube.
6. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

## Addition of Complexes to Cells

1. Add the transfection complex drop-wise, to the 6-well plates containing cells in complete growth medium (cell seeding step). Swirl plate gently after each addition.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the GeneXPlus Transfection Reagent:DNA complexes.
3. Incubate for 24 to 72 hours. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay as required.

**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.

**Large-scale transfections:** Table 1 recommends starting volumes for 20-mL transfections (125-mL shaker flask). The volumes listed can be directly scaled up in proportion to the culture volume, if larger volumes are desired.

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## Quality Control Specifications

**Functional tests:** Functional Assay

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: GeneXPlus Transfection Reagent (ATCC ACS-4004)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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## Revision

This information on this document was last updated on 2022-11-05

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