ATCC

Product Sheet

GeneX*Plus* Transfection Reagent (ATCC® ACS-4004™)

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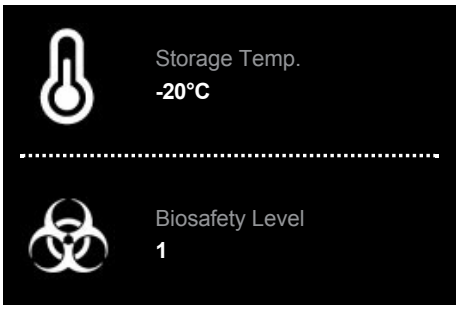

Description

Product Description: GeneX*Plus* Transfection Reagent is a high-performance, animal-origin free, 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. GeneX*Plus* works effectively in large scale reactions, producing high protein yields. Since the reagent is composed of animal-origin free components and is serum compatible, there is no need for any culture medium change after transfection.
GeneX*Plus* Transfection Reagent has been optimized for use with HEK293T/17 SF suspension cells (ATCC ACS-4500) and HEK*Plus* SFM (ATCC ACS-4004) to reproducibly express a wide range of proteins at high levels.
Volume: 1 mL

Directions for Use

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 GeneX*Plus* 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 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 1:4. A DNA (µg) to reagent (µL) ratio of 1:3 is recommended as a starting point.

4. Complex formation conditions. Prepare GeneX*Plus* 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 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.

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Storage Temp.
-20°C

Biosafety Level
1

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	10-cm dish	T75 flask	125-mL shaker flask
Surface area (cm^2)	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) (µL)	9	50	100	250	1500	1900	2000
Amount of DNA (µg)	0.1	0.5	1	2.5	15	19	20
Transfection reagent (µL)	0.3	1.5	3	7.5	45	57	60

Transient plasmid DNA transfection protocol per well of a 6-well plate
***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 > 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 x 10^5 to 6 x 10^5 cells/well.

   For suspension cells: Plate cells at a density of 6 x 10^5 to 8 x 10^5 cells/mL.
2. Incubate cell cultures overnight.

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 250 µL of serum-free complete growth medium in a sterile tube.
3. Add 2.5 µg (2.5 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 7.5 µL GeneX*Plus* 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 GeneX*Plus* 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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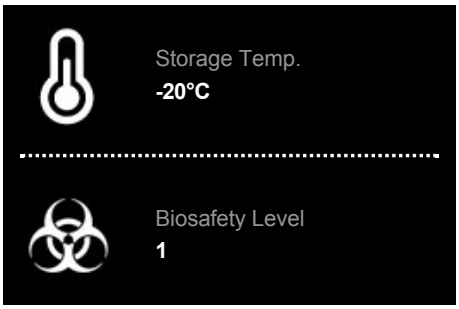

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