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Contact & Technical support

Tel.: 0731 3608 123
Fax: 0731 3608 962
e-mail: info@genaxxon.com

Genaxxon bioscience GmbH
Sölflinger Str. 70
D-89077 Ulm

www.genaxxon.com

GenaxxoFect Transfection Kits

Protocol for GenaxxoFect and GenaxxoFect-*plus* Transfection Kits

Cat#: M3053
Cat#: M3055

Version: 110117

2. General Information about GenaxxoFect Reagents

2.1 Characteristics of GenaxxoFect and GenaxxoFect-*plus*

GenaxxoFect and **GenaxxoFect-*plus*** are suitable for small and large scale transfection of DNA.

GenaxxoFect and **GenaxxoFect-*plus*** are ideal for high throughput cell based screens.

GenaxxoFect is a reagent with especially low cytotoxicity.

GenaxxoFect-*plus* is suited for transfection of cells that are difficult to transfect. **GenaxxoFect-*plus*** is an optimized formulation requiring less reagent per transfection.

2.2 Handling of GenaxxoFect Transfection Reagents

GenaxxoFect Reagents should be mixed by vortexing before each use. **Do not aliquot and store GenaxxoFect Reagents** in containers other than the one it is delivered in as contact of undiluted liposomal reagents with plastic surfaces may reduce performance. Dilute by pipetting GenaxxoFect Reagents directly into the supplied Dilution Buffer, avoiding contact with the side of tubes, and pipette action to wash out traces remaining in the pipette tip.

GenaxxoFect and **GenaxxoFect-*plus*** are easy to use, serum compatible and free of animal derived components. There is no need for medium change after transfection. **GenaxxoFect** reagents have relatively low cytotoxicity, allowing easy One-Step* cell transfection or recentyl detached cells. This One-Step procedure reduces the duration of your experiment by one day. Once diluted, **GenaxxoFect** and **GenaxxoFect-*plus*** can be usded for a period of up to four days. Always mix the dilution directly before use.

Properties of GenaxxoFect Transfection Reagents

- Small / large scale
- High Throughput
- Serum compatible
- No medium change
- No dangerous components
- Less DNA necessary / normally <50ng (<2ng for plasmids)
- Transfection efficiencies >80% (HEK, PAC2)
- Very easy to use

* One-Step transfection: Transfection method in which cell plating and transfection is performed in one single step (see also section 3.2.1, page 2).

Transfection of neuronal cells

Tests conducted by Genaxxon and customers of Genaxxon with transfection of neuronal cells are not finished yet but these results show that the GenaxxoFect-products can be used successfully.

Our own experiments on transfection of *Neuro2a* cell lines show that **GenaxxoFect-*plus*** gives the best result regarding efficiency and viability.

We found the following conditions as optimal for this cell line (in 96-well plates):

GenaxxoFect-*plus*:	0.15µL/well
Nucleic acid:	75ng/well

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Trouble shooting (continued)

Possible cause	Suggestions
Inappropriate cell density or cells are not actively dividing	70-90% confluence is recommended for most cell lines at time of transfection.
	The cells used for transfection should be in exponential growth phase and have even density over the entire surface area. Ensure appropriate density of your cell culture by timely passaging. This is important for both One- and Two-Step transfection protocols.
	For the Two-Step method, split your cell culture early enough before transfection (15 – 24 h should be appropriate).
	Excessive passaging of cells decreases the transfection performance so use cells with similar passage number between experiments to ensure reproducibility.
	Use a new batch of cells.
Inhibition of complex formation	Serum does not affect the performance of **GenaxxoFect Reagents** but we recommend avoiding antibiotics during transfection as well as anionic inhibitors such as EDTA or dextran sulphate.
Improper protocol	We highly recommend the One-Step transfection method for all our reagents! Based on our experience, One-Step transfection leads to increased performance of **GenaxxoFect Reagents** compared to Two-Step.
	The time-saving One-Step transfection method may not be suited for all cell types and applications. Only if One-Step transfection does not lead to the desired results test the Two-Step method: Cells are plated into the desired format the day before so that they are adherent at time of transfection. Shortly before the addition of transfection complexes to adherent cells, we recommend removing all (for weakly adherent cells, half) of the medium from the cells. In case of 96-well plates, 80µL of fresh medium is then mixed with the transfection complexes and this mixture is then added to the adherent cells. Take care to avoid cell detachment, especially for weakly adherent cells.
Incorrect vector	Make sure the promoter on the vector being used works with the cell type in your experiment.
	Verify the DNA sequence
	Use GFP plasmid to monitor transfection efficiency by fluorescence microscopy.

3. Basic considerations for successful transfection

3.1. Cells

Cells used for transfection should be mycoplasma free (simple PCR test), in exponential growth phase and have relatively even density over the entire surface area on which they are plated. We recommend splitting cells when they reach 80% - 90% confluence to avoid contact inhibition of cell proliferation.

3.2 Transfection Methods

Cells can be transfected using the two different methods described below.

3.2.1. "One-Step" method (combined plating + transfection)

For One-Step transfection (also referred to as Reverse Cell Transfection), freshly detached* cells in suspension are added to the transfection complexes. The transfection process is thus initiated before cell attachment takes place.

Important facts and benefits of One-Step transfection:

- Time efficient procedure (combined plating and transfection)
- Highly recommended for GenaxxoFect Reagents
- Due to the low cytotoxicity of the reagents, reverse cell transfection ("One-Step2 method) does not harm the cells, but …
- Significantly increases transfection efficiencies for most cell lines tested.

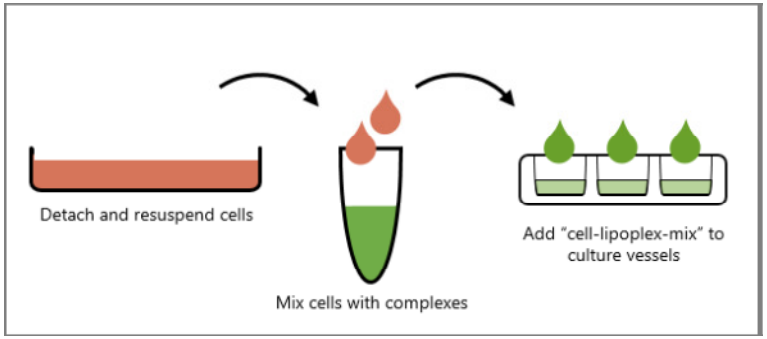

Figure 1: One-Step transfection method

** Detachment of cells using Accutase (cat#: C4355) is highly recommended. Accutase was developed for gentle and effective detachment of adherent cells and does not cleave cells surface protein like Trypsin does, meaning faster adhering time and recovery of cells after plating. It costs about the same as Trypsin. Try it and see!*

3.2.2. "Two-Step" transfection (Forward Cell Transfection)

In the Two-Step or Forward Transfection Method, the cells are plated 24 hours before transfection. The next day, complexes of transfection reagent and nucleic acid are added to the already adherent cells. For more information see page 17 "Troubleshooting".

The one-step method is easiest and saves time, however some cell types are more efficiently transfected as already adherent monolayers. Whatever method is chosen, it is important to use cells that are/have been neither too densely nor too sparsely growing. We recommend splitting cells when they reach 80% - 90% confluence to avoid contact inhibition of cell proliferation.

3.3 Nucleic Acid

For best transfection results, nucleic acid should be pure and endotoxin-free. An A_{260}/A_{280} absorption ratio of 1.7 to 1.9 is recommended for plasmid DNA (pDNA).
The amount of any particular pDNA construct required per transfection is dependent on the gene itself, the promoter driving expression of the gene and the plasmid backbone. Therefore, it is important to determine the appropriate amount of nucleic acid per transfection through optimization experiments (see section 4.4.2, page 14).

3.4. GenaxxoFect reagent dilution and complex formation

Transfection complex formation is a critical step for optimal transfection results. The nucleic acid and the transfection reagent must be evenly mixed in Dilution Buffer, both as their separate dilutions as well as when subsequently combined for transfection complex formation. IF previously dilutes **GenaxxoFect Reagents** are to be used again (up to 4 days storage possible) mix by vortexing immediately before addition of DNA.
For optimal mixing at onset of transfection complex formation, equal volumes of nucleic-acid- and **GenaxxoFect Reagent** dilutions are combined using fast pipette action to rapidly from a homgenious mixture. Strong vortexing is not recommended during complex formation due to the strong shear forces that may disrupt the complex formation process. For larger scale transfections (e.g. transfection in culture dishes) "splitting" larger volumes into smaller aliquots for the complex formation step is recommended. Ensure at least 20 minutes of transfection complex formation time for optimal results.

3.5 Serum and Antibiotics

Serum does not affect the performance of GenaxxoFect Reagents. Although there is no clear evidence for a reduced transfection efficiency using antibiotics, we recommend avoiding Penicillin and Streptomycin during transfection, especially for siRNA transfection.

5. Troubleshooting

In case of low transfection efficiency, use the troubleshooting guide below as a basis to identify the problem.

Possible cause	Suggestions
Poor quality of DNA or Insufficient DNA amount	Check the quality and concentration of plasmid DNA. Ideally, the ratio A_{260}/A_{280} is approximately 1.8.
	The DNA used for transfection should be free of any kind of contamination. Contaminations like RNA and proteins may influence the transfection efficiency.
	Optimise the concentration of DNA according to the initial optimisation protocol in section 4.4.2.
	Make sure the DNA which is used for transfection is endotoxin free.
Insufficient complex formation	Make sure the diluted **GenaxxoFect Reagent** and nucleic acid solutions are, after combination, mixed immediately using rapid pipette action. **Do not vortex!** (see section 3.4 on page 5).
	If the volume used for complex formation is greater than 200µL, it may help to split the complex formation step into several tubes of reduced volumes – in order to make the initial mixing step more efficient (see section 3.4 on page 6-7).
	Incubate the prepared pDNA and **GenaxxoFect Reagent** for at least 20 minutes at RT.
Improper GenaxxoFect Reagent to DNA ratio	Optimize the DNA-to-GenaxxoFect Reagent ratio according to the optimization protocol in section 4.4.2.
Incorrect Storage	**GenaxxoFect Reagents** should be stored at +2°C to +8°C. Do not leave for extended periods of time at RT and do not freeze. Try to avoid excessive warming-cooling if used frequently by users. Remove from 4°C for brief periods when needed and replace at 4°C.
Cells are unhealthy	The cells used for transfection should be mycoplasma free, in exponential growth phase and have even density over surface area. Make sure your cells are free of any contamination. The use of antibiotics is recommended during passaging. Ensure the density of your cell culture does not get too high or too low during the experiment and while passaging cells – always maintain timely passaging.
	Perform a negative control / cells-alone control experiment to verify the cell health.

4.4.2.1 Optimization of pDNA and siRNA transfections in selected formats

The following table is intended to assist the user with optimization of both pDNA and siRNA transfections in different formats. Only the volume ranges of reagent, dilution buffer and the ranges of nucleic acid amounts are included into the table below. For operational instructions, please refer to section 4.4.2.

Nucleic Acid	**pDNA**			**siRNA**		
Plate format	96	24	6	96	24	6
Step 1	Dilute **GenaxxoFect Reagent** in Dilution Buffer					
GenaxxoFect Reagent	0.35µL	1.7L	6µL	0.45µL	2µL	4.5µL
Total volume of dilution	10µL	40µL	120µL	10µL	40µL	120µL
Step 2	Dilute nucleic acid in Dilution Buffer					
Nucleic acid	75ng	300ng	1000ng	1pmol	10pmol	30pmol
Total volume of dilution	10µL	40µL	120µL	10µL	40µL	120µL
Step 3	Add diluted **GenaxxoFect Reagent** to the nucleic acid dilutions and mix with pipette.					
Volume of mixture	20µL	80µL	240µL	20µL	80µL	240µL
Step 4	After 20 min add freshly resuspended cells (or medium in case of Two-Step) to complexes and transfer mixture to cell culture plate.					
Cell suspension	80µL	420µL	1250µL	80µL	420µL	1250µL

4.1 Overview: How to use GenaxxoFect and GenaxxoFect-*plus*

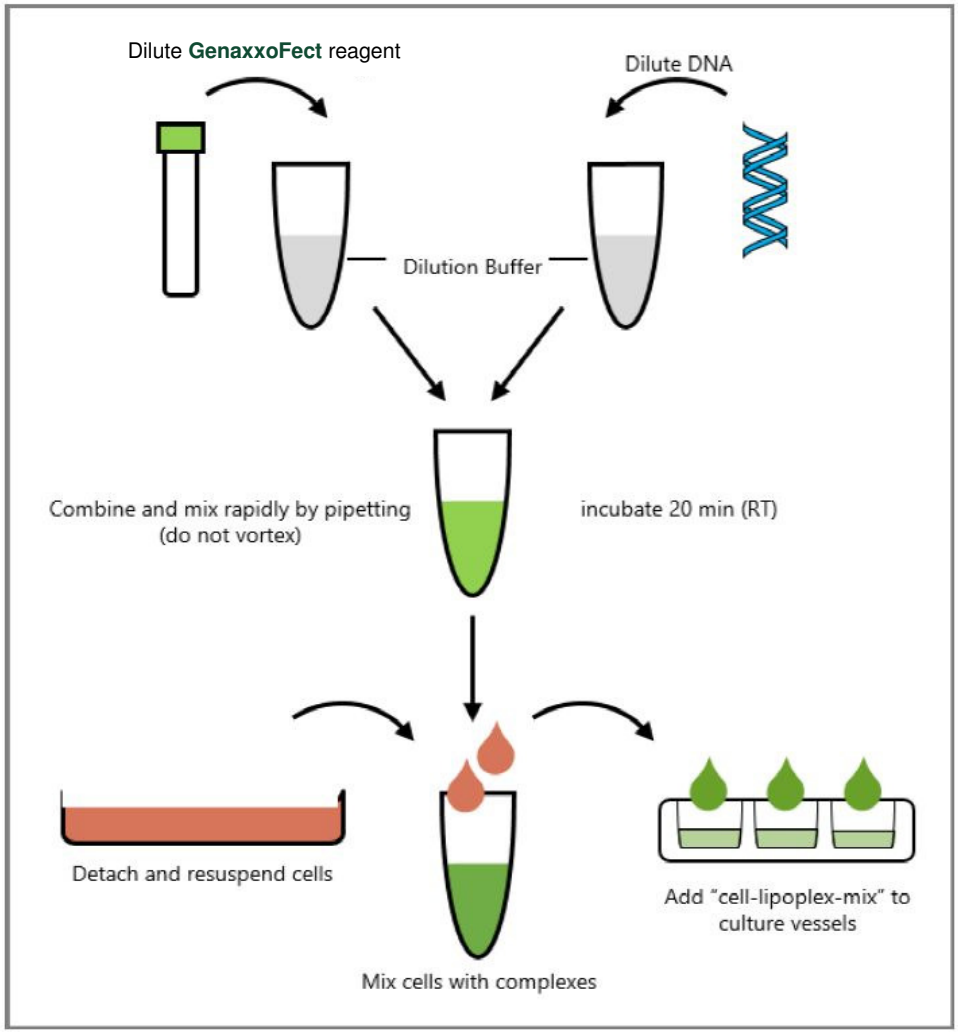

Figure 2: GenaxxoFect transfection procedure at a glance

4.4.2 Optimizing GenaxxoFect and GenaxxoFect-*plus* plasmid DNA Transfection

1.	**Prepare 3 different plasmid DNA dilutions**
1A	Prepare 3 different 50µL samples of plasmid DNA* taking a total of 250ng, 375ng, 500ng and diluting to **50µL** Dilution Buffer each. ***Important:*** *We recommend using a pDNA mixture consisting of 10% GFP and 90% of an empty vector for a first optimization, as GFP is eaily monitored by fluorescence microscopy. Optimal transfection conditions are strongly dependent on the pDNA construct that is used. Therefore, if your target construct differs significantly (e.g. in size) from GFP pDNA, use your target pDNA and the corresponding assay for optimization.*
1B	Take 4 x 10µL from each of the above 3 DNA samples and distribute between the wells of a 96-well PCR plate as shown in the pipetting scheme section 4.4.2, page 15 (10µL of diluted reagent will be added to these 12 samples later). ***NOTE:*** *The DNA samples can alternatively be distributed between tubes of PCR-stripes.*
2.	**Prepare 4 different dilutions of GenaxxoFect or GenaxxoFect-*plus* transfection reagent:**

Prepare the following 4 **GenaxxoFect or GenaxxoFect-*plus*** dilutions in PCR strips. Mix by rapid pipette action or vortexing.

Sample	Corresponding final volume of GenaxxoFect Reagent per 96-well	Volume of Dilution Buffer	Volume of GenaxxoFect or GenaxxoFect-*plus*
2.1	0.1µL	198µL	2µL
2.2	0.2µL	98µL	2µL
2.3	0.3µL	97µL	3µL
2.4	0.4µL	96µL	4µL

≈

Important: add **GenaxxoFect Reagent** *directly into supplied buffer with rapid pipette mixing, or vortexing without touching the sides of tubes with tip submerged in buffer.*

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4.4.2 Optimizing GenaxxoFect and GenaxxoFect-*plus* Plasmid DNA Transfection (continued)

3.	**Mix each diluted GenaxxoFect sample with the different DNA samples:**
	Add 10µL of each diluted **GenaxxoFect Reagent** sample to each of the three different amounts of DNA in a PCR-reaction tube, mix and incubate 20 min. at RT.
	Take 10µL aliquots from the 4 **GenaxxoFect Reagent** dilutions and add to the DNA samples containing 50ng (samples **1-4** for plate coordinates C5-C8 as show below). Immediately mix with 10 rapid pipette strokes, **do not vortex!** Repeat for the DNA samples containing 75ng (**samples 5-8**) and 100ng (**samples 9-12**).

Pipetting scheme

pDNA amount	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
50ng C					**1**	**2**	**3**	**4**				
75ng D					**5**	**6**	**7**	**8**				
100ng E					**9**	**10**	**11**	**12**				
F												
G												
H												

µL of GenaxxoFect Reagent: 0.1 / 0.2 / 0.3 / 0.4
per well (and sample ID)*: 2.1 2.2 2.3 2.4

*** pipetting scheme example on page 14**

4.	Mix transfection complexes with cells:
	The One-Step tranfection method gives best results. Add 80µL freshly dissociated cells to the complexes and then mix gently with pipette before transferring to a 96-well cell culture plate.
	For the ***Two-Step*** *method, first remove the used medium from the 96-well cell culture plate of already adherent cells. Then add 80µL medium to transfection complexes and transfer medium and complexes to plate with adherent cells.*

Note: View next page for information regarding the optimization of pDNA and siRNA transfections in different formats.