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GenaxxoFect Transfection Kits

Product information and instruction Manual for GenaxxoFect and GenaxxoFect-*plus* Transfection Kits

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Cat#: M3055

Version: 170117

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- PCR / RT-PCR
- Transfection Reagents
- DNA and RNA Purification
- Molecular Biology Products
- Proteins and Peptides
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Related Products

Product	Contents	Cat. No.
GenaxxoFect siRNA	0.2mL 1.0mL 10.0mL	M3050.0002 M3050.0001 M3050.0010
GenaxxoFect	0.2mL 1.0mL 10.0mL	M3053.0002 M3053.0001 M3053.0010
GenaxxoFect-*plus*	0.2mL 1.0mL 10.0mL	M3055.0002 M3055.0001 M3055.0010
GenaxxoFect mRNA	0.2mL 1.0mL 10.0mL	M3059.0002 M3059.0001 M3059.0010
D-PBS solution w/o NaHCO **D-PBS solution (10-times) w/o NaHCO3**	500mL 500mL	C4217.0500 C4218.0500
PBS solution w/o Mg, Ca, NaHCO3 **PBS solution (10-times) w/o Mg, Ca, NaHCO3**	500mL 500mL	C4219.0500 C4220.0500
Pen-Strep	100mL 50x1mL	M3140.0100 M3140.5001
DMEM Medium		Ask for details
RPMI Medium		Ask for details
Cytokines and Growth Factors		Ask for details
96-well semi-skirted Microtiter plates	25 plates 100 plates	I2014.0025 I2014.0100
PCR DNA Purification Mini Prep Kit	50 preps 250 preps	S5368.0050 S5368.0250

Hotline: +49 731 3608 123 or info@genaxxon.com

Safety Information

GenaxxoFect and **GenaxxoFect-*plus*** do not contain dangerous components and are not flammable, so no special safety measures are required. Avoid contact with eyes and skin and observe the normal laboratory safety precautions when handling, i.e. wearing protective gloves, goggles and clothing. In case of accident, note the following first aid measures:

Skin contact: Instantly wash with water and soap and rinse thoroughly.
Eye contact: Rinse opened eye for several minutes under running water. Consult doctor.
If swallowed: Seek immediate medical advice.

Please see Material Safety Data Sheet (MSDS) for more detailed information!

Storage Conditions and Stability

GenaxxoFect and **GenaxxoFect-*plus*** should be stored at 4°C. Do not leave for extended periods at room temperature and do not freeze. For optimal long term activity, do not allow **GenaxxoFect or GenaxxoFect-*plus*** to warm to room temperature (RT) each time it is used - remove from 4°C only for short periods when required for dilution in the provided buffer. If kept refrigerated at 4°C **)**

Shipping

Shipment is done at ambient temperature to reduce environmental waste and cost. We see no loss of activity with storage at room temperature for up 2 weeks at RT.

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Manual Contents

Subject	Page
1. Introduction	1
2. General Information about GenaxxoFect Reagents	3
2.1 Characteristics of GenaxxoFect and GenaxxoFect-*plus*	3
2.2 Handling of GenaxxoFect Transfection Reagents	3
3. Basic considerations for Successful Transfection	4
3.1 Cells	4
3.2 Transfection Methods	4
3.2.1 One-Step Transfection (combined plating & transfection)	4
3.2.2 Two-Step Transfection (forward cell transfection)	5
3.3 Nucleic Acid	5
3.4 GenaxxoFect Reagent Dilution and Complex Formation	5
3.5 Serum and Antibiotics	5
4. GenaxxoFect and GenaxxoFect-*plus* Transfection Protocols	6
4.1 Overview: How to use GenaxxoFect and GenaxxoFect-*plus*	7
4.2 GenaxxoFect Transfection Protocols	
4.2.1 GenaxxoFect plasmid DNA Transfection Protocol	8
4.2.1.1 Transfection of plasmid DNA in 96-well format	8
4.2.1.2 Tabular transfection protocol for selected formats	9
4.3 GenaxxoFect-*plus* Transfection Protocols	10
4.3.1 GenaxxoFect-*plus* plasmid DNA Transfection Protocol	10
4.3.1.1 Transfection of plasmid DNA in 96-well format	10
4.3.1.2 Tabular transfection protocol for selected formats	11
4.4 Optimization Protocol for GenaxxoFect and GenaxxoFect-*plus*	12
4.4.1 Impact of pDNA-to-Reagent-Ratio and total amount of DNA or reagent on cell transfection results.	12
4.4.2 Optimizing GenaxxoFect and GenaxxoFect-*plus* plasmid DNA transfection	14
4.4.2.1 Optimization of pDNA and siRNA transfections in selected formats	16
5. Troubleshooting	17
6.1. Transfection protocol for transfection with miRNA	19
Transfection of neuronal cells (*Neuro2a* cell line)	20
Limited License – Limitations of product use – Quality Control	22
Safety Information – Storage Conditions - Shipping	23

1. Introduction

GenaxxoFect is a transfection reagent that was selected after various cell based screening experiments using hundreds of novel lipid-like chemicals that were synthesized using a unique chemistry. GenaxxoFect products are **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 of recently detached cells. This One-Step procedure reduces the duration of your experiment by one day. Once diluted, GenaxxoFect and GenaxxoFect-plus can be used for a period of up to four days. Always mix the dilution directly before use.

GenaxxoFect is highly efficient for DNA transfection and has low cellular toxicity. It is highly efficient with common cell lines as well as “difficult to transfect” cells such as mouse embryonic stem cells (mESC) and zebrafish PAC2 cells.

List of cells successfully transfected with **GenaxxoFect ***

DNA	**DNA**	**DNA**	**DNA**
143BTK cell line	786-O_HKC	A431	A375 / A2058
COS-7	H9C2	H9C2	HEK293 (suspension)
HEK293	HeLa	HEP-2	HepG2
HUVEC	Ins-1	L4-2B_Du14S	LO2
mC3T3	MDCK	MEF	mES
mHSC	NIH 3T3	PLC8024	RAW264.7_MEF_U937
SKOV3	MTPa	PAC2	mESC
siRNA	**siRNA**	**siRNA**	**siRNA**
HEK293 TN	HEP-2	HepG2	DB lymphoma cell line
M3CT3-E1	MCF-10A/HeLa		
others	**others**	**others**	**others**
HEK293 TN	SK-Hep1	LO2	HL7704_BEL7402_THP-1
MCF7			

* this list is not comprehensive. GenaxxoFect can transfect a wide range of different cells. Ask Genaxxon for more details. Or just try is out. Samples are available at Genaxxon.

Limited License

The purchase price paid for the **GenaxxoFect** and **GenaxxoFect-*plus* Kit** by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended for internal research only by the purchaser. Furthermore, research only use means that **GenaxxoFect** and **GenaxxoFect-*plus* Kit** and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without written approval of the manufacturer.

Separate licenses are available from the manufacturer for the express purpose of non-research use and applications. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, please contact your local distributor.

Limitations of Product Use

The use of this kit is strictly limited to research purposes. They are not to be applied for any diagnostic, including human, or drug purposes. This also excludes administration to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the regulatory authorities in the country of use. All due care and attention should be exercised in handling of the materials described in this handbook.

Before using a **GenaxxoFect** or **GenaxxoFect-*plus* Kit**, customers and other users should make their own determination that the product is suitable for intended use. They should ensure that they can use the **GenaxxoFect** or **GenaxxoFect-*plus* Kit** safely and legally. This document does not constitute a warranty or assume any liabilities on behalf of the manufacturer except in writing signed by the manufacturer. Unless otherwise agreed in writing, the exclusive remedy for all claims is replacement of the product or refund of the purchase price at manufacturer’s option, and in no event shall the manufacturer be liable for special, consequential, incidental, punitive or exemplary damages.

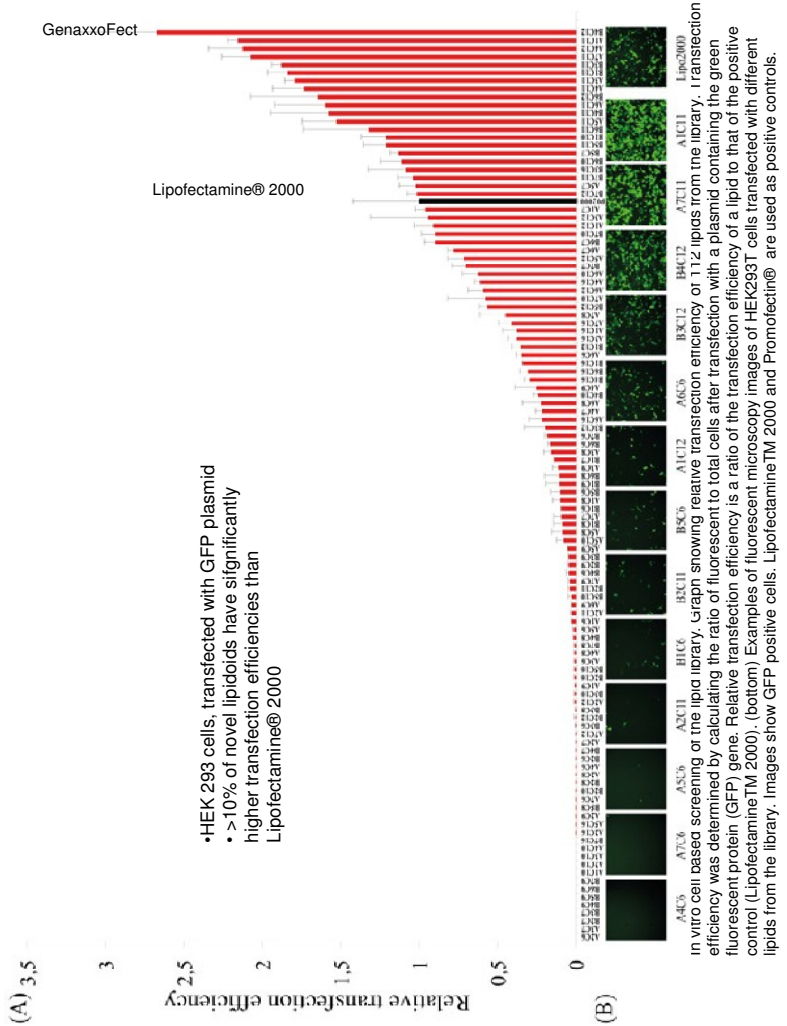
Quality Control

Genaxxon bioscience is dedicated to your success and every batch of this product is tested with an extensive routine procedure to make sure that it meets all your needs. However, it has neither been developed nor tested for a specific application.

Genaxxon reserves the right to change, alter, or modify the **GenaxxoFect** or **GenaxxoFect-*plus* Kit** to enhance its performance and design.

This product is for research use only.

Notes:



In vitro cell based screening of the lipid library. Graph showing relative transfection efficiency of 112 lipids from the library. Transfection efficiency was determined by calculating the ratio of fluorescent to total cells after transfection with a plasmid containing the green fluorescent protein (GFP) gene. Relative transfection efficiency is a ratio of the transfection efficiency of a lipid to that of the positive control (Lipofectamine TM 2000). (bottom) Examples of fluorescent microscopy images of HEK293T cells transfected with different lipids from the library. Images show GFP positive cells. Lipofectamine TM 2000 and Promofectin® are used as positive controls.

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 4).

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

6.1 GenaxxoFect or GenaxxoFect siRNA protocol for microRNA (miRNA)
6.1.1 Transfection of adherent cells in 96-well format.

	Amounts shown are for **one 96-well**.
1	Prepare 1µM miRNA solution in sterile RNase-free water. Pipette 5.5µL of the miRNA solution into wells of a V-bottomed 96-well plate or PCR tube strips.
2	Dilute between 0.2 and 0.4µL of **GenaxxoFect** or **GenaxxoFect siRNA** to a final volume of 5.5µL in Dilution Buffer.
3	Combine solution 1 and 2 by pipetting 5.5µL diluted **GenaxxoFect reagent** into each V-bottomed well or tube containing 5.5µL of miRNA. Cover carefully with sealing film, vortex briefly (1s!), centrifuge briefly and leave transfection complexes to form for 30 min at RT.
	Important: *Pipette the diluted GenaxxoFect reagent to the diluted RNA and immediately mix with one pipette stroke. After covering, vortex for 1s, not longer! Centrifuge to collect lipoplexes at bottom of all well/tubes.*
4	Remove old medium from adherent cells in 96-well culture plate. Add 99µL of fresh medium (containing 10% FBS) to each 11µL of transfection complexes, mix once with pipette and transfer 100µL of this solution immediately to the cells.
	Tip*: Try to work quickly to ensure the time between removing the "old" medium from cells and addition of fresh medium containing the transfection complexes is kept to a minimum.*
5	After 3-6 hours, add a further 75µL of pre-warmed medium (containing 10% FBS) to the transfected cells and incubate till end point analysis.
	Note: *Addition of additional fresh medium after transfection helps to reduce cellular toxicity. It is usually not necessary to replace the medium the next day.*

Note: *Serum does not affect the performance of* **GenaxxoFect** *or* **GenaxxoFect siRNA** *but we recommend avoiding Pen/Strep. Cells must be mycoplasma free, in exponential growth phase and have even plating density over the entire surface area.*

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.

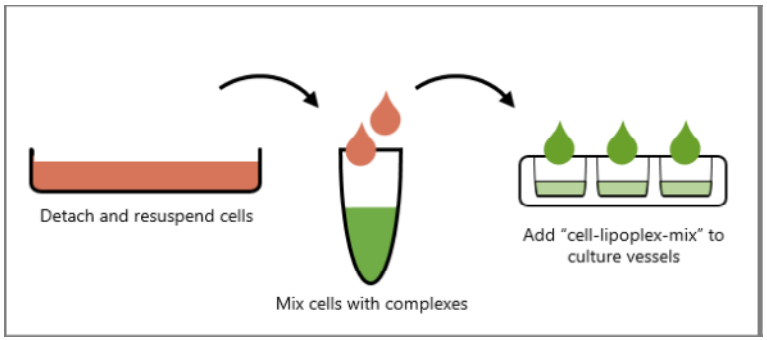


Figure 1: One-Step transfection method

** 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.

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.

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. GenaxxoFect and GenaxxoFect-*plus* DNA Transfection Protocols

The following pages contain protocols for **GenaxxoFect** and **GenaxxoFect-*plus*** transfection. The reagent volumes suggested in the protocols of sections 4.2 and 4.3 are a guideline. Initial optimization is important. Therefore, an optimization protocol is provided at the end of this chapter. The optimization protocol helps users to determine the optimal amount of **GenaxxoFect** or **GenaxxoFect-*plus*** reagent as well as nucleic acid for their particular transfection experiment and cell line.
We highly recommend the One-Step transfection protocol for all our GenaxxoFect products. However, for special applications or cell types, the Two-Step protocol may be preferable. Advices on how to use our reagents in Two-Step transfections are given in section 5, page 18 Troubleshooting.

Transfection of neuronal cell lines (see page 20).

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

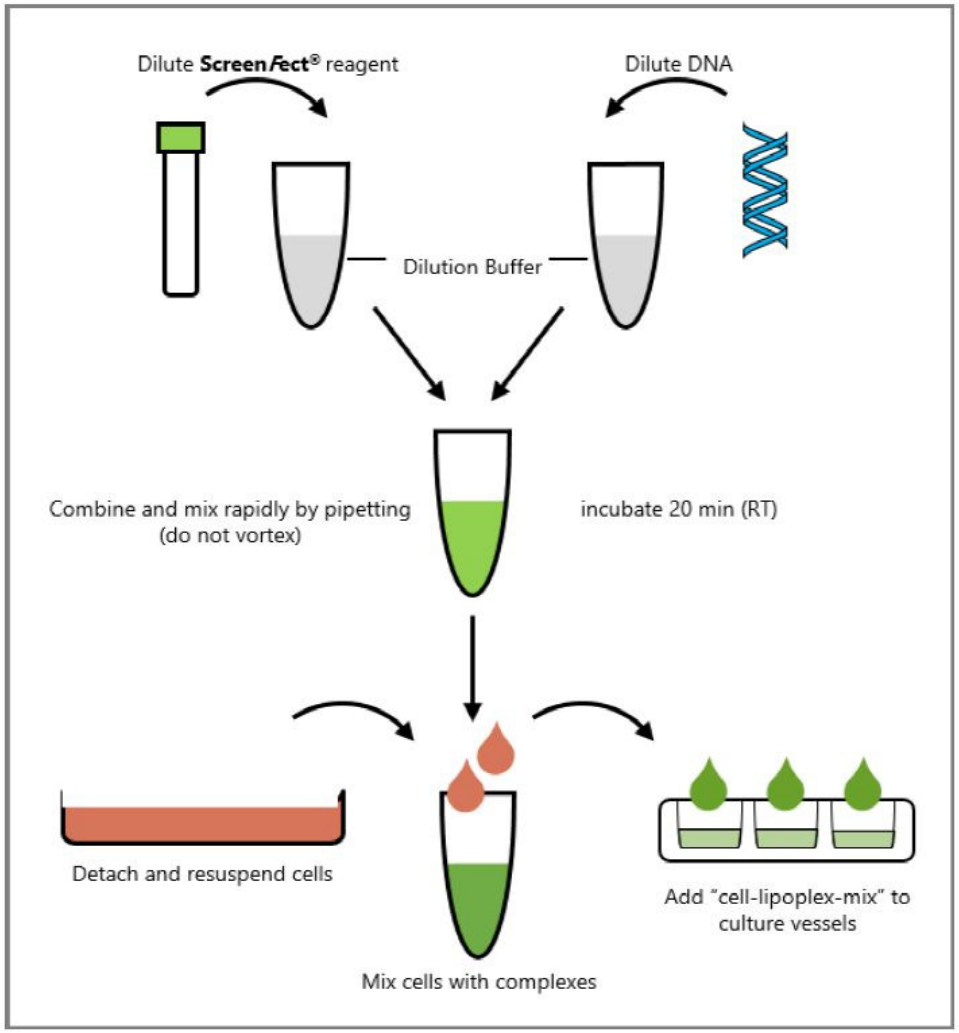


Figure 2: GenaxxoFect transfection procedure at a glance

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

4.4.2 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.

4.2 GenaxxoFect Transfection Protocols

4.2.1 GenaxxoFect plasmid DNA Transfection Protocol

4.2.1.1 Transfection of plasmid DNA in 96-well format.

	Amounts shown are for **one 96-well**.
1	Dilute 0.35µL* of **GenaxxoFect** in supplied Dilution Buffer, to a final volume of 10µL and mix thoroughly (at RT).
	***Important**: Vortex the reagent before use. Add **GenaxxoFect** reagent directly into supplied buffer with rapid pipette mixing or vortexing.*
2	Dilute a **total** of **75ng*** plasmid DNA in supplied Dilution Buffer to a final volume of 10µL.
	***Tip**: include a positive control for quick and easy detection of transfection (e.g. GFP plasmid to monitor transfection efficiency by fluorescence microscopy).*
3	Combine the diluted **GenaxxoFect** and DNA and mix **immediately** using **10 rapid pipette strokes.** Allow complex formation to proceed for 20 min at RT.
	Important**: **Do not vortex!
4	Add **80µL** freshly detached and resuspended cells to complexes (enough for a 60-80% confluent monolayer next day) and mix gently with pipette.
	***Tip**: The time saving reverse transfection method may not be suited for all cell types (see section 4.1, page 7). To transfect already adherent cells (60-80% confluent), first remove and discard medium from cells, then add 80µL fresh culture medium to transfection complexes, mix with pipette and immediately apply to cells.* *use Accutase (cat#: C4355) rather than Trypsin-EDTA to detachment of cells*
5	Transfer cells and complexes to one well of a **96-well** plate.

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.4.2 (page 15). This is intended to help the user to determine the optimal amount of both GenaxxoFect as well as pDNA for transfection of the particular cell type within one experiment.

***Note:** Serum does not affect the performance of **GenaxxoFect** but we recommend avoiding Pen/Strep. Cells must be mycoplasma free, in exponential growth phase and have even plating density over the entire surface area.*

4.2.1.2 Tabular transfection protocol for selected formats (DNA transfection)

For formats other than 96-well, scale reagents using the table below as a guideline.

	96-well (1x)	**24-well** (~5x)	**12-well** (~8x)	**6-well** (~15x)
	step 1: dilute **GenaxxoFect** in Dilution buffer			
GenaxxoFect	0.35µL	2µL	4µL	6µL
Dilution Buffer	10µL	40µL	80µL	120µL
	step 2: dilute plasmid DNA in Dilution buffer			
Plasmid DNA (total)	~75ng	~300ng	~600ng	~1000ng
Supplied Dilution Buffer	10µL	40µL	80µL	120µL
	step 3: add diluted **GenaxxoFect** to DNA-dilution rapidly and mix with rapid pipette action			
	20µL	80µL	160µL	240µL
	step 4: after 20 min. add freshly resuspended cells (or medium) to transfection complexes and transfer mixture to plate			
Cell suspension (or medium)	~80µL	~350µL	~700µL	~1250µL

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.4.2 (page 15). This is intended to help the user to determine the optimal amount of both GenaxxoFect as well as pDNA for transfection of the particular cell type within one experiment.

4.2.2 siRNA Transfection Protocol

NOTE: Although **GenaxxoFect and GenaxxoFect-*plus*** work well for siRNA transfection, for optimal results we highly recommend the use of our specialized reagent GenaxxoFect siRNA (see M3050).

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.*

In order to find the best ration and concentration of pDNA and reagent for your cell line and application, you need to consider the following:

- Higher amounts of pDNA usually result in higher expression of the recombinant protein.
- Low concentration of pDNA and reagent result in mild conditions for the cells and therefore in low cell toxicity.
- **Transfection efficiency and cell toxicity negatively correlate with each other.** It is essential to find working conditions at which these characteristics are balanced. **Be sure to choose robust conditions** ("optimal working range" in the picture below) that are not sensitive to experimental deviations.

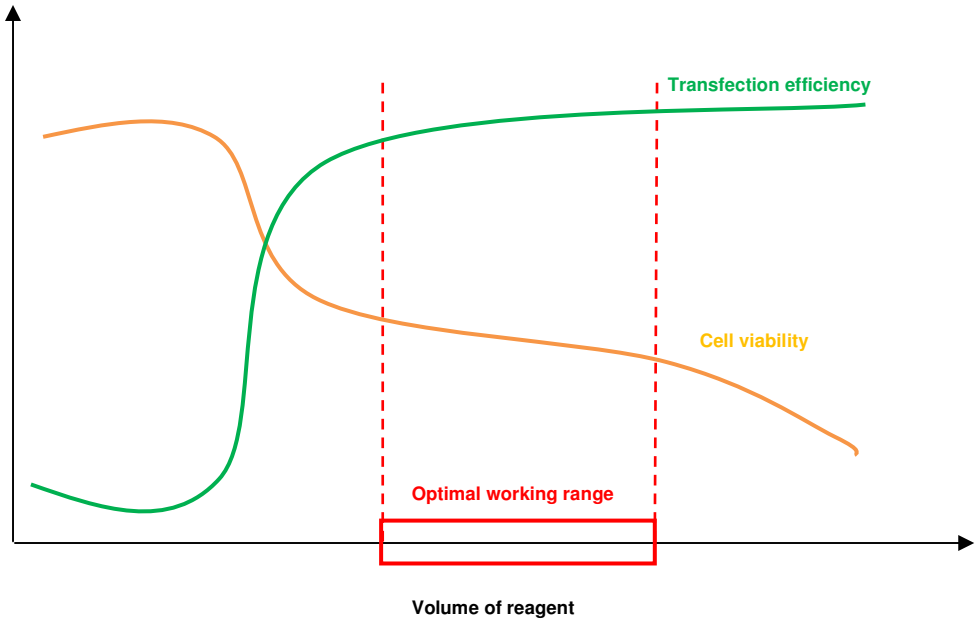


Figure 3: General correlations between reagent volumes, transfection efficiency and cell viability.

4.3 GenaxxoFect-*plus* Transfection Protocols

4.3.1 GenaxxoFect-*plus* plasmid DNA Transfection Protocol

4.3.1.1 Transfection of plasmid DNA in 96-well format.

	Amounts shown are for **one 96-well**.
1	Dilute 0.15µL* of **GenaxxoFect-*plus*** in supplied Dilution Buffer, to a final volume of 10µL and mix thoroughly (at RT).
	Important**: Vortex the reagent before use. Add **GenaxxoFect-*plus reagent directly into supplied buffer with rapid pipette mixing or vortexing.*
2	Dilute a **total** of **75ng*** plasmid DNA in supplied Dilution Buffer to a final volume of 10µL.
	***Tip**: include a positive control for quick and easy detection of transfection (e.g. GFP plasmid to monitor transfection efficiency by fluorescence microscopy).*
3	Combine the diluted **GenaxxoFect** and DNA and mix **immediately** using **10 rapid pipette strokes.** Allow complex formation to proceed for 20 min at RT.
	Important**: **Do not vortex!
4	Add **80µL** freshly detached and resuspended cells to complexes (enough for a 60-80% confluent monolayer next day) and mix gently with pipette.
	***Tip**: The time saving reverse transfection method may not be suited for all cell types (see section 4.1, page 7). To transfect already adherent cells (60-80% confluent), first remove and discard medium from cells, then add 80µL fresh culture medium to transfection complexes, mix with pipette and immediately apply to cells.* *use Accutase (cat#: C4355) rather than Trypsin-EDTA to detachment of cells*
5	Transfer cells and complexes to one well of a **96-well** plate.

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.4.2 (page 15). This is intended to help the user to determine the optimal amount of both GenaxxoFect as well as pDNA for transfection of the particular cell type within one experiment.

Note:** Serum does not affect the performance of **GenaxxoFect-*plus but we recommend avoiding Pen/Strep. Cells must be mycoplasma free, in exponential growth phase and have even plating density over the entire surface area.*

4.3.1.2 Tabular transfection protocol for selected formats (DNA transfection)

For formats other than 96-well, scale reagents using the table below as a guideline.

	96-well (1x)	**24-well** (~5x)	**12-well** (~8x)	**6-well** (~15x)
	step 1: dilute **GenaxxoFect-*plus*** in Dilution buffer			
GenaxxoFect	0.15µL	1µL	2µL	4µL
Dilution Buffer	10µL	40µL	80µL	120µL
	step 2: dilute plasmid DNA in Dilution buffer			
Plasmid DNA (total)	~75ng	~300ng	~600ng	~1000ng
Supplied Dilution Buffer	10µL	40µL	80µL	120µL
	step 3: add diluted **GenaxxoFect** to DNA-dilution rapidly and mix with rapid pipette action			
	20µL	80µL	160µL	240µL
	step 4: after 20 min. add freshly resuspended cells (or medium) to transfection complexes and transfer mixture to plate			
Cell suspension (or medium)	~80µL	~350µL	~700µL	~1250µL

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.4.2 (page 15). This is intended to help the user to determine the optimal amount of both GenaxxoFect as well as pDNA for transfection of the particular cell type within one experiment.

4.3.2 siRNA Transfection Protocol

NOTE: Although **GenaxxoFect and GenaxxoFect-*plus*** work well for siRNA transfection, for optimal results we highly recommend the use of our specialized reagent GenaxxoFect siRNA (see M3050).

4.4 Optimization Protocol for GenaxxoFect and GenaxxoFect-*plus*

Optimizing transfection conditions is highly recommended for new users to ensure optimal transfection results. Here we provide a basic protocol for varying both the amount of GenaxxoFect Reagent as well as plasmid DNA (pDNA) in one convenient experiment.

4.4.1 Impact of pDNA-to-Reagent-Ratio and Total Amounts of DNA or Reagent on Cell Transfection Results

The following pictures show microscope images of GFP expressing cells 24h after transfection. The optimization protocol (section 4.4.2, page 15) was strictly followed. A 96-well format was chosen for transfection.
The result show that the reagent volume range of efficient cell transfection changes with changing amounts of pDNA used per well.

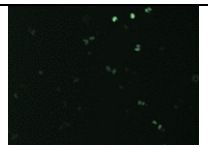
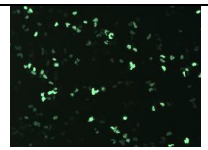
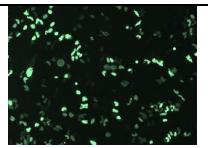
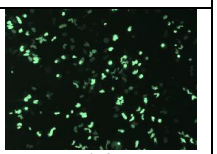
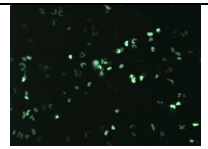
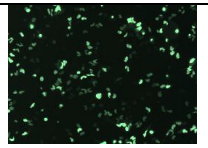
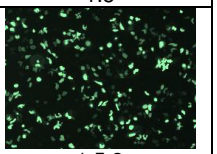
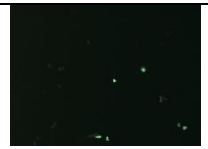
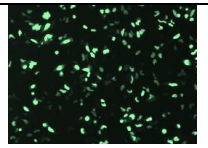
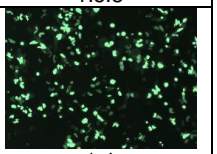
reagent / pDNA	0.1µL	0.2µL	0.3µL	0.4µL
50ng				
ratio	1:2	1:4	1:6	1:8
75ng				
ratio	1:1.3	1:2.7	1:4	1:5.3
100ng				
ratio	1:1	1:2	1:3	1:4

Table 1: Fluorescent microscope images of GFP expression cells.
The amount of pDNA was varied from 50 to 100ng. Volumes of reagent used per well were varied between 0.1 and 0.4µL.