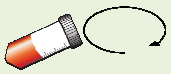
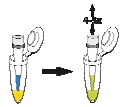
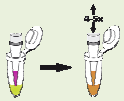
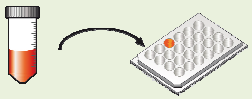
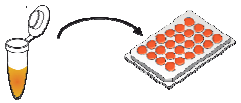
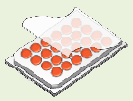
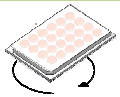
Transfection protocol for plasmid DNA (pDNA) delivery into suspension cells using GenaxxoFect reagents:

	Transfection Protocol for suspension cells with GenaxxoFect reagents in 24-well plate:
	**1.** Seed cells one day prior to transfection so that they reach a maximum cell density of 1.0 – 3.0 x 10^6 viable cells per mL at the time of transfection.
	**2.** **On day of transfection**: Cells are counted and cell viability is determined (**Note:** cell viability must not be < 90% and viable cell density should not exceed 1.5 x 10^6 cells per mL).
 130 g 3 min	**3.** Centrifuge the cells for 3 min at 130 x g (780 rpm) and resuspend an appropriate amount of cells in fresh medium (Cap-T Express or PEM) to a final cell density of **4.0 x 10^5 viable cells per mL.**
	**4.** For each transfection (each well), add **2-4 µl of GenaxxoFect®** or derivatives transfection reagent into **100 µl GenaxxoFect® Dilution Buffer** in a sterile microfuge tube and mix with a few pipette strokes.
	**5.** Add **0.3 - 0.5 µg pD2EGFP** or other expression plasmid per tube and mix well with pipette strokes.
	**6.** Incubate at room temperature for **25 minutes** for lipoplex formation.
	**7.** Add **1ml of prepared cell suspension** to each well of a **24-well plate** and make sure that the solution is homogenously mixed.
	**8.** **Add the transfection mixture** sequentially (drop wise) to each well.
	**9.** **Seal the plate** with a sterile BreathSeal® gas permeable sealing foil and put the lid back on top of the plate.
 37°C 48h – 72h	**10.** **Incubate** at 37°C, CO_2, 85% humidity with agitation at 180 rpm (25 mm orbit) for 48h to 72h in an orbital shaker incubator.

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Quick Protocol for GenaxxoFect-plus

Protocol for pDNA Transfection

	Component	Procedure for one well (96-well-plate)	96-well	24-well	6-well
1.	**Reagent Dilution**	Dilute 0.15µL of GenaxxoFect-plus in Diution Buffer to a final volume of 10µL and mix thoroughly	0.15µL reagent 10µL dilution	1µL reagent 40µL dilution	4µL reagent 120µL dilution
		Important: Vortex the reagent once per day of use. Add GenaxxoFect-plus reagent directly into supplied buffer with rapid pipette mixing or vortexing.			
2.	**pDNA Dilution**	Dilute a total 75ng pDNA in Dilution Buffer to a final volume of 10µL.	75ng 10µL dilution	300ng 40µL dilution	1000ng 120µL dilution
		Tip: Include a positive control for quick and easy detection of transfection (e.g. using GFP plasmid and fluorescence microscopy).			
3.	**Complex formation**	Combine the diluted GenaxxoFect-plus and DNA and mix immediately using 10 rapid pipette strokes. Leave for 20 minutes at room temperature for complex formation.	20µL complexes	80µL complexes	240µL complexes
		Important: Do not vortex!			
4.	**Cell preparation & transfection**	Add 80µL freshly detached and resuspended cells to the complexes (enough cells to result in a 60-80% confluent monolayer on the following day) and mix with pipette.	Add 80µL cell suspension	Add 420µL cell suspension	Add 1250µL cell suspension
		Tip: The time-saving reverse cell transfection method may not be suited for all cell types. To transfect 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.			
5.	**Cell plating**	Transfer the cells and complexes to one well of a 96-well plate.	Transfer cells with complexes to plate	Transfer cells with complexes to plate	Transfer cells with complexes to plate

Note: This protocol is a guideline. Values are suitable for easy for transfect cell lines. This protocol does not replace optimization experiments. View our manual for instructions.
Serum does normally not affect the performance of GenaxxoFect-plus but we recommend avoiding at least antibiotics.
Cells must be mycoplasma free, in exponential growth phase and have even plating density across the entire surface area.