Xpert Transfection Reagent

#GTC50.0001 (1ml)

(FOR RESEARCH ONLY)

Product: High performance multi-purpose reagent with very low cytotoxicity, suitable for the transfection of most cell lines, including stem cells, using either pDNA, siRNA, mRNA or miRNA.
Xpert Transfection Reagent is serum compatible and free of animal derived components, hence there is no need to change the medium after transfection.

Quantity: #GTC50.0001 comprises 1ml of Xpert Transfection Reagent. Depending on the application (pDNA, siRNA, mRNA or miRNA) and the plate size (6-wells, 24-wells or 96-wells), one ml of Xpert Transfection Reagent is sufficient for 150 to 5000 transfections (see protocols)

Contents:

Product	GTC50.0001
Xpert Transfection Reagent	1 ml
Dilution Buffer	50 ml

Applications: pDNA transfection
mRNA transfection
miRNA transfection
siRNA transfection

Properties: High efficiency with low toxicity Serum compatible and free of animal components
Excellent performance with primary cell lines and stem cells

Storage: Stable at +4°C (2-8°C) for at least 1 year. During long time storage, slight precipitation may occur, which has no effect on performance. Do not freeze. Do not prepare aliquots as this may lead lose to loss of activity as undiluted liposomal reagents bind to plastic. Once diluted, Xpert transfection reagent can be used up for a period of 3 days. Mix well before each usage.

Xpert Transfection Reagent can be shipped at without cooling. There is no significant loss of performance with storage at room temperature for periods up to 2 weeks.

Prior to use:
Negatively charged nucleic acid (NA) combines with the cationic lipids present in Xpert Transfection Reagent to form so-called transfection complexes. This process is formation is dependent on the DNA-to-liposome ratio. For optimal results, the basic protocols should be optimized for each cell type and for each NA, regarding amounts and ratio of Xpert Transfection Reagent and NA.

Moreover, for optimal results, nucleic acids should be pure and endotoxin-free, thus ensure the quality and quantity of your RNA samples. For plasmid DNA (pDNA), an A_{260nm}/A_{280nm} ratio of 1.7-1.9 is recommended. It has been demonstrated that, supercoiled plasmid forms more efficient transfection complexes than relaxed pDNA, due to differences in compactness.

Despite lack of evidence, it is recommended to avoid usage of penicillin and streptomycin during transfection as there are indications that these antibiotics affect transfection negatively, especially in the case of siRNA. It is also recommended to avoid the usage of anionic inhibitors, such as EDTA and dextran sulfate.

Basic Protocols

(These basic protocols serve as general guidelines and are sub-optimal. Protocols for optimization experiments can be found on our website)

Prior to transfection, a transfection complex is formed by mixing Xpert Transfection Reagent with the nucleic acid of choice. Subsequently, adherent cells are detached, resuspended and mixed with the transfection complex, and finally transferred to plates.

1. pDNA transfection

Step	Procedure	6-well plate	24-well plate	96-well plate
1. Xpert Transfection Reagent dilution	Dilute transfection reagent directly in Dilution Buffer *)	6 µl reagent +114 µl buffer (120 µl total)	2 µl reagent +33 µl buffer (35 µl total)	0.35 µl reagent +7.65 µl buffer (8 µl total)
2. pDNA dilution	Dilute transfection reagent directly in Dilution Buffer **)	1 µg pDNA +buffer (120 µl total volume)	300 ng pDNA +buffer (35 µl total volume)	75 ng pDNA +buffer (8 µl total volume)
3. Transfection Complex Formation	Combine both dilutions (step 1+2) mix by pipetting up and down***)	Incubate 20 min at RT	Incubate 20 min at RT	Incubate 20 min at RT
4. Preparation of Cells and Transfection	Add freshly detached and resuspended cells to the combined mixture.	Add 1250 µl of cell suspension and mix by pipetting	Add 420 µl of cell suspension and mix by pipetting	Add 80 µl of cell suspension and mix by pipetting
5. Cell plating	Transfer cells mixed with transfection complex to plates	Transfer to a 6-well plate and incubate	Transfer to a 24-well plate and incubate	Transfer to a 96-well plate and incubate

*) Vortex the Transfection Reagent on the day of use. Pipet directly in dilution buffer and mix thoroughly by pipetting up and down
**) It is recommended to include a positive control that allows for fast and easy detection of transfection (such as GFP plasmid)
***) Do not vortex

2. siRNA transfection

Step	Procedure	6-well plate	24-well plate	96-well plate
1. Xpert Transfection Reagent dilution	Dilute transfection reagent directly in Dilution Buffer *)	4.5 µl reagent +115.5 µl buffer (120 µl total)	2 µl reagent +33 µl buffer (35 µl total)	0.45 µl reagent +7.55 µl buffer (8 µl total)
2. siRNA dilution	Dilute transfection reagent directly in Dilution Buffer	30 pmol siRNA +buffer (120 µl total volume)	10 pmol siRNA +buffer (35 µl total volume)	1 pmol siRNA +buffer (8 µl total volume)
3. Transfection Complex Formation	Combine both dilutions (step 1+2) mix by pipetting up and down***)	Incubate 20 min at RT	Incubate 20 min at RT	Incubate 20 min at RT
4. Preparation of Cells and Transfection	Add freshly detached and resuspended cells to the combined mixture.	Add 1250 µl of cell suspension and mix by pipetting	Add 420 µl of cell suspension and mix by pipetting	Add 80 µl of cell suspension and mix by pipetting
5. Cell plating	Transfer cells mixed with transfection complex to plates	Transfer to a 6-well plate and incubate	Transfer to a 24-well plate and incubate	Transfer to a 96-well plate and incubate

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***) Do not vortex

3. mRNA transfection

Step	Procedure	6-well plate	24-well plate	96-well plate
1. Xpert Transfection Reagent dilution	Dilute transfection reagent directly in Dilution Buffer *)	6 µl reagent +114 µl buffer (120 µl total)	2 µl reagent +33 µl buffer (35 µl total)	0.35 µl reagent +7.65 µl buffer (8 µl total)
2. mRNA dilution	Dilute transfection reagent directly in Dilution Buffer	1 µg mRNA +buffer (120 µl total volume)	300 ng mRNA +buffer (35 µl total volume)	75ng mRNA +buffer (8 µl total volume)
3. Transfection Complex Formation	Combine both dilutions (step 1+2) mix by pipetting up and down***)	Incubate 20 min at RT	Incubate 20 min at RT	Incubate 20 min at RT
4. Preparation of Cells and Transfection	Add freshly detached and resuspended cells to the combined mixture.	Add 1250 µl of cell suspension and mix by pipetting	Add 420 µl of cell suspension and mix by pipetting	Add 80 µl of cell suspension and mix by pipetting
5. Cell plating	Transfer cells mixed with transfection complex to plates	Transfer to a 6-well plate and incubate	Transfer to a 24-well plate and incubate	Transfer to a 96-well plate and incubate

*) Vortex the Transfection Reagent on the day of use. Pipet directly in dilution buffer and mix thoroughly by pipetting up and down
***) Do not vortex

4. miRNA transfection

Step	Procedure	6-well plate	24-well plate	96-well plate
1. Xpert Transfection Reagent dilution	Dilute transfection reagent directly in Dilution Buffer *)	4.5 µl reagent +115.5 µl buffer (120 µl total)	2 µl reagent +33 µl buffer (35 µl total)	0.45 µl reagent +7.55 µl buffer (8 µl total)
2. miRNA dilution	Dilute transfection reagent directly in Dilution Buffer	30 pmol miRNA +buffer (120 µl total volume)	10 pmol miRNA +buffer (35 µl total volume)	1 pmol miRNA +buffer (8 µl total volume)
3. Transfection Complex Formation	Combine both dilutions (step 1+2) mix by pipetting up and down***)	Incubate 20 min at RT	Incubate 20 min at RT	Incubate 20 min at RT
4. Preparation of Cells and Transfection	Add freshly detached and resuspended cells to the combined mixture.	Add 1250 µl of cell suspension and mix by pipetting	Add 420 µl of cell suspension and mix by pipetting	Add 80 µl of cell suspension and mix by pipetting
5. Cell plating	Transfer cells mixed with transfection complex to plates	Transfer to a 6-well plate and incubate	Transfer to a 24-well plate and incubate	Transfer to a 96-well plate and incubate

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