Xpert Transfection Reagent
Optimization Protocol

In general, higher concentrations of nucleic acid (e.g., pDNA or siRNA) lead to higher transfection efficiency (leading to higher recombinant protein expression or more efficient gene silencing). However, lower concentrations of nucleic acids and reagent lead to milder conditions for the cells and thus in lower cell toxicity. There is a clear negative correlation between transfection efficiency and cell toxicity and it is essential to determine the conditions that favor a balanced transfection process.

The Basic Protocols mentioned in the GTC50 Xpert Transfection Reagent product information datasheet serve as general guidelines. To ensure the most efficient transfection, initial optimization is important. Below, we present an optimization protocol for plasmid DNA, which serves to determine the optimal amounts and ratio of Xpert Transfection Reagent and pDNA, as they vary with the nature of the cell line and nucleic acid.

Optimization of pDNA transfection using 96-well plate

1) Dilute 3 (or more) different amounts (e.g., 250ng, 375ng, 500ng) of plasmid DNA in Dilution Buffer, to a final volume of 50µl (for each).

2) Prepare 4 different dilutions of Xpert Transfection Reagent in Dilution Buffer as shown in the table below

Dilution	Dilution Buffer (µl)	Xpert Transfection Reagent (µl)	Equivalent final vol (per well) (µl)
1	198	2	0.1
2	98	2	0.2
3	97	2	0.3
4	96	2	0.4

3) Pipet 10µl of each plasmid mixture in 4 wells of a 96-well plate, as shown in the scheme hereunder.
(In case of having prepared 250ng of plasmid DNA in 50µl Dilution Buffer, pipetting 10µl into well C4, C5, C6 and C7 corresponds to 50ng of plasmid DNA in each of these wells as shown in this example, and so on)

	1	2	3	4 (D.1)	5 (D.2)	6 (D.3)	7 (D.4)	8	9	10	11	12
A												
B												
C				50	50	50	50					
D				75	75	75	75					
E				100	100	100	100					
F												
G												
H												

4) Add 10µl of each of the Xpert Transfection Reagent Dilutions to each of the plasmid samples, as shown in the scheme above. (In this case, add 10µl of Dilution 1 to C4, D4, and E4, 10µl of Dilution 2 to C5, D5 and E5, and so on)

5) Incubate at room temperature for 20 minutes

6) Add 80µl of cell suspension and mix by pipetting 5 times up and down. Do not vortex.

7) Evaluate transfection efficiency after 24 hours.

This protocol can be easily adapted to other amounts of pDNA, to other plate formats (e.g., 6-well or 24-well) and to other types of nucleic acids (mRNA, siRNA, miRNA). We suggest to start with making the following adaptions to the protocol. Note: the amounts are per well.

pDNA transfection

Step	per well	6-well	24-well	96-well
1	plasmid DNA (ng)	600, 1000, 1400	200, 300, 400	50, 75, 100
	plasmid Volume (µl)	120	30	10
2	Xpert Transfection Reagent (µl)	4.0, 5.0, 6.0	1.0, 1.5, 1.7	0.1, 0.2, 0.3, 0.4
	Volume Reagent after Dilution (µl)	120	30	10
-	(total Volume (µl))	240	60	20
6	Cell suspension (µl)	1250	420	80

siRNA transfection

Step	per well	6-well	24-well	96-well
1	siRNA (pmol)	20, 40, 60	10, 15, 20	1.0, 2.0, 3.0
	siRNA Volume (µl)	120	30	10
2	Xpert Transfection Reagent (µl)	3.0, 4.5, 6.0	0.75, 1.4, 2.0	0.25, 0.35, 0.45
	Volume Reagent after Dilution (µl)	120	30	10
-	(total Volume (µl))	240	60	20
6	Cell suspension (µl)	1250	420	80

mRNA transfection

Step	per well	6-well	24-well	96-well
1	mRNA (ng)	600, 1000, 1400	200, 300, 400	50, 75, 100
	mRNA Volume (µl)	120	30	10
2	Xpert Transfection Reagent (µl)	4.0, 5.0, 6.0	1.0, 1.5, 1.7	0.25, 0.35, 0.45
	Volume Reagent after Dilution (µl)	120	30	10
-	(total Volume (µl))	240	60	20
6	Cell suspension (µl)	1250	420	80

miRNA transfection

Step	per well	6-well	24-well	96-well
1	miRNA (pmol)	20, 40, 60	10, 15, 20	1.0, 2.0, 3.0
	miRNA Volume (µl)	120	30	10
2	Xpert Transfection Reagent (µl)	3.0, 4.5, 6.0	0.75, 1.4, 2.0	0.25, 0.35, 0.45
	Volume Reagent after Dilution (µl)	120	30	10
-	(total Volume (µl))	240	60	20
6	Cell suspension (µl)	1250	420	80