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SI-XpressFect

Catalog Number	Size
SIXF10-1	SI-XpressFect Transfection Reagent: 1.0 ml 10X SI Buffer: 2.0 ml
SIXF10-2	SI-XpressFect Transfection Reagent: 2 x 1.0 ml 10X SI Buffer: 2 x 2.0 ml
SIXF10-5	SI-XpressFect Transfection Reagent: 5 x 1.0 ml 10X SI Buffer: 5 x 2.0 ml

Description:

SI-XpressFect is a composition of lipids especially developed for the transfection of mammalian cells with siRNA or miRNA and generates exceptional knockdown. It enables transfection to be performed with extremely small amounts of reagent, resulting in excellent cell viability and minimizing off-target effects. Additionally, SI-XpressFect can be used with a fast forward protocol enabling two consecutive experiments to be performed per week (standard protocol with 48-hour incubation time) and is ideal for automated laboratory testing.

Application: Transfection of nucleic acids into mammalian cells with siRNA or miRNA

Formulation: Composition of lipids in water

Assays: Up to 4000 (96-well) or about 650 (24-well) with 1 ml reagent

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like SI-XpressFect change their size distribution after long storage at +4°C, which can have slight adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. It is recommended to perform a freeze-thaw cycle before first use, and subsequently monthly to yield optimal results.

Note: This product is for research use only. Not for use in human or animal diagnostics, therapeutics, or clinical applications.

General Guidelines

State of Cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as

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cells which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination with mycoplasma or fungi can drastically alter transfection results and must be excluded.

Quality of the Nucleic Acid

The RNA should be of maximum purity if optimal knockdown results are desired. RNA is sensitive to ubiquitous RNases, appropriate precautions should be taken.

Transfection Protocol

Notes

The following protocol describes transfection in which cells in two wells of a 48-well plate (each with 1 square centimeter growth area) are treated with two different quantities of an RNA/lipid complex (lipoplex). Following the protocol is a chart of quantities for other well formats.

In the fast-forward protocol cells are seeded shortly before the addition of lipoplex. If using sensitive cells, we recommend incubating overnight after seeding.

The experiment is then evaluated to give optimum amount of lipoplex for the cells. All further experiments involving the same calls and the same RNA use this optimum amount of lipoplex in transfection. Amounts for these explicit transfection protocols are given in the **Explicit Transfection Protocols** section.

In rare cases (e.g. highly sensitive or hard to transfer cells), optimization of the RNA:lipid ratio may be useful. See the **Advanced Optimization** section.

Reagent Preparation

1. SI Buffer – Make 1X SI Buffer from the provided 10X SI Buffer. To do this, mix 1 part 10X SI Buffer with 9 parts of sterile water suitable for cell culture under sterile conditions.
2. Prior to transfection, bring 1X SI Buffer, SI-XpressFect Transfection Reagent and RNA stock solution (concentration of at least 20 µM = 20 pmol/µl) to room temperature. Agitate all reagents gently before use.

Cell Preparation

1. Prepare 500 µl of cell suspension with at a concentration of 2.0 x 10^5 cells/ml (1.5 – 2.5 x 10^5 cells/ml) in complete cell culture medium.
2. Fill two wells of a 48-well plate (well 1 and well 2) with 250 µl of cell suspension each.
3. Incubate cells under normal cell culture conditions (e.g. 37°C in CO_2 containing atmosphere) until the lipoplex is added. If using sensitive cells, it is recommended to incubate overnight after seeding.

Lipoplex Preparation

1. Place 45 µl 1X SI Buffer in the test tube (ideally polypropylene).
2. Pipette 2.4 µl SI-XpressFect Transfection Reagent into the 1X SI Buffer and mix the solution by gently pipetting up and down once.

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3. Add 90 pmol RNA by pipetting and mix gently once.
   Note: Mix gently, shearing forces damage the lipoplex and reduce transfection efficiency.
4. Incubate for 15 minutes at room temperature.

Transfection

1. Add the lipoplex solution to the wells of cell suspension as follows:

   Well 1: 15 µl of lipoplex (contains ~ 30 pmol RNA)

   Well 2: 30 µl of lipoplex (contains ~ 60 pmol RNA)

2. Mix the solutions in both wells by gently pipetting up and down once.
3. Incubate the wells under normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Evaluation

Evaluation generally takes place 24 – 72 hours after the addition of lipoplex. The highest knockdown is often achieved after 48 hours. The optimal point in time is determined by the properties of the cell type and the expression rate of the protein as well as by the half-life of the expressed protein (if applicable).

Use the results to select the optimum amount of lipoplex (well 1 or well 2) for the cells used.

Conversion to Other Wells Formats

Format	Area	Cell suspension	1X SI Buffer	SI-XpressFect Transfection Reagent	RNA	Lipoplex volume	
						Well 1	Well 2
96–well	0.3 cm^2	2× 100 µl	22.5 µl	0.72 µl	27 pmol	7.5 µl	15 µl
48–well	1.0 cm^2	2× 250 µl	45 µl	2.4 µl	90 pmol	15 µl	30 µl
24–well	1.9 cm^2	2× 500 µl	90 µl	4.6 µl	170 pmol	30 µl	60 µl
12–well	3.6 cm^2	2× 900 µl	180 µl	8.6 µl	320 pmol	60 µl	120 µl
6–well	9.0 cm^2	2× 2.2 ml	450 µl	21.6 µl	810 pmol	150 µl	300 µl
60 mm dish	22 cm^2	2× 5.5 ml	1350 µl	53 µl	2.0 nmol	450 µl	900 µl
100 mm dish	60 cm^2	2× 15 ml	4.0 ml	144 µl	5.4 nmol	1.35 ml	2.7 ml

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Explicit Transfection Protocols

If the optimum amount of lipoplex (well 1 or well 2) for the cells to be transfected is already known, the following chart can be used as a basis for further procedure.

Amounts given refer to transfection of a **single** well with the given format. The process of the lipoplex preparation is the same as described in the **Lipoplex Preparation** section.

	Lipoplex volume 1 (Well 1)			**Lipoplex volume 2 (Well 2)**		
Format	**1X SI Buffer**	**SI-XpressFect Transfection Reagent**	**RNA**	**1X SI Buffer**	**SI-XpressFect Transfection Reagent**	**RNA**
96–well	7.5 µl	0.24 µl	9 pmol	15 µl	0.48 µl	18 pmol
48–well	15 µl	0.8 µl	30 pmol	30 µl	1.6 µl	60 pmol
24–well	30 µl	1.5 µl	57 pmol	60 µl	3.0 µl	114 pmol
12–well	60 µl	2.9 µl	108 pmol	120 µl	5.8 µl	216 pmol
6–well	150 µl	7.2 µl	270 pmol	300 µl	14.4 µl	540 pmol
60 mm dish	450 µl	17.6 µl	660 pmol	900 µl	35.2 µl	1.3 nmol
100 mm dish	1.35 ml	48 µl	1.8 nmol	2.7 ml	96 µl	3.6 nmol

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Advanced Optimization

Adjustment of the RNA:lipid ration is generally unnecessary in transfection with SI-XpressFect. However, changes to transfection parameters may be useful in the case of hard-to-transfect or highly sensitive cells.

The following chart shows all useful combinations of reagent amount and RNA amount for the various well formats. Standard amounts are underlined.

	Lipoplex amount 1					Lipoplex amount 2				
Format	**1× SI Buffer**	**SI-XpressFect Transfection Reagent (µl)**			**RNA**	**1× SI Buffer**	**SI-XpressFect Transfection Reagent (µl)**			**RNA**
96 Well	7.5 µl	0.12	0.24	0.36	9 pmol	15 µl	0.24	0.48	0.72	18 pmol
48 Well	15 µl	0.40	0.80	1.2	30 pmol	30 µl	0.8	1.6	2.4	60 pmol
24 Well	30 µl	0.76	1.5	2.3	57 pmol	60 µl	1.5	3.0	4.6	114 pmol
12 Well	60 µl	1.45	2.9	4.3	108 pmol	120 µl	2.9	5.8	8.6	216 pmol
6 Well	150 µl	3.6	7.2	10.8	270 pmol	300 µl	7.2	14.4	21.6	540 pmol
60 mm Dish	450 µl	8.8	17.6	26.4	660 pmol	900 µl	17.6	35.2	52.8	1.3 nmol
100 mm Dish	1.35 ml	24	48	72	1.8 nmol	2.7 ml	48	96	144	3.6 nmol