



X-tremeGENE siRNA Transfection Reagent

For the transfection of siRNA into animal cells

Cat. No. 04 476 093 001

1 ml

Cat. No. 04 476 115 001

Multi-pack 5 × 1 ml (2000 transfection)

Version June 2006

Store at +2 to +8°C

1. What this Product Does

Number of Transfection Experiments

Using standard experimental conditions, one milliliter of X-tremeGENE siRNA Transfection Reagent transfects HeLa, NIH 3T3, Hek 293 cells, or other mammalian cells in over four hundred wells of a 24-well plate. This is equivalent to over 1,600 transfections in 96-well plates.

Contents

Formulation X-tremeGENE siRNA Transfection Reagent is a proprietary blend of lipids and other components supplied in aqueous solution, sterile-filtered, and packaged in polypropylene tubes. The reagent is free of any components derived from animals.

Storage and Stability

X-tremeGENE siRNA Transfection Reagent is shipped at room temperature and is stabilized for extended storage at +2 to +8°C through the expiration date printed on the label when tightly closed.

⚠ Always mix X-tremeGENE siRNA Transfection Reagent prior to use (vortex for one second or invert). Do not freeze!

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to transfection experiments.

- Prewarmed sterile, serum-free culture medium without additives or supplements. Opti-MEM-1 Medium is recommended for dilution of the siRNA and the X-tremeGENE siRNA Transfection reagent.
- Mammalian cell line of interest
- siRNA solution between 0.2 µg/µl (15 pmoles/µl, *i.e.* 15 µM) and 2 µg/µl (150 pmoles/µl, *i.e.* 150 µM) in sterile buffer.

Application

X-tremeGENE siRNA Transfection Reagent is a multicomponent reagent that forms a complex with siRNA, and then delivers it into animal cells.

Features of X-tremeGENE siRNA Transfection Reagent include:

- High transfection efficiency in many common cell types, including HeLa, NIH 3T3, Hek 293, PC-3 and COS-7.
- The use of one single reagent for siRNA- and cotransfection-based RNA experiments.
- Low cytotoxicity, the change of media after the addition of transfection complex is not required.
- Functions exceptionally well in the presence or absence of serum; eliminates the need to change media.

2. How To Use this Product

2.1 Before You Begin

siRNA purity, fluorescently labeled siRNAs

Determine the siRNA purity using a 260 nm/280 nm ratio; the ratio should be 1.9 or higher. When using siRNA preparations from *in vitro* transcription reactions, the size of less than 30 basepairs of the siRNA needs to be carefully controlled, as non-embryonic animal cells show an interferon response resulting in a general translation block with larger dsRNA species (1). A recent publication (1) describes that the 5' tri-phosphate residues of *in vitro* transcribed siRNA must be removed to avoid an interferon response. Chemically synthesized siRNAs from commercial suppliers are usually sufficiently pure for transfection.

⚠ Use fluorescently labeled siRNAs: The uptake or non uptake of fluorescently labeled siRNAs does not necessarily correlate with the knockdown. Even with no visible uptake of labeled siRNAs, successful knockdown was demonstrated.

Cell-Culture Conditions

Minimize both intra- and inter-experimental variance in transfection efficiency by using cells that are regularly passaged, proliferating well (best when in a loggrowth phase), and plated at a consistent density.

Effect of Media and Media Additives, including Sera

- ① In some cell types, antimicrobial agents (*e.g.*, antibiotics and fungicides) that are commonly included in cell culture media may adversely affect the transfection efficiency of X-tremeGENE siRNA Transfection Reagent. If possible, exclude additives for initial experiments. Once high-efficiency conditions have been established, these components can be added back while monitoring your transfection results.
- ② Different media and media components may influence the level of transfection efficiency and subsequent growth of the transfected cells, as well as knockdown of the gene of interest.
- ③ Any medium can be used for cell cultivation and during transfection. However, Opti-MEM-1 Medium is recommended for dilution of the siRNA and the X-tremeGENE siRNA Transfection reagent (see Procedure).
- ③ Test different media and optimize the level of each medium component for these effects. Although it is not usually necessary to remove the transfection reagent : siRNA complex following the transfection step, it is necessary to feed your cells with fresh media for extended growth periods. This is particularly important when the transfected cells are allowed to grow for 3–7 days for maximal knockdown with very stable proteins.

Verification of Transfection Efficiency

As the knockdown efficiency is dependent on the gene as well as on the cell line, determination of the transfection efficiency is recommended when using a new cell line. The knockdown of the endogenous human HPRT housekeeping gene can be measured with Q-PCR. The knockdown of the endogenous lamin A/C gene with anti-lamin A/C antibodies (BD Biosciences) was demonstrated in western blots.

Incubation Time

Incubate the cells for 24–72 hours. The length of incubation depends upon the siRNA, the cell type being transfected, the stability of the mRNA, or the protein being targeted. After this incubation period, measure the knockdown using an assay that is appropriate for your system.

2.2 Procedure

Preparation of Cells for Transfection

One day prior to the transfection experiment, trypsinize, adjust the cell concentration, and plate the cells in the chosen cell culture vessel. For most cell types, plating $0.1 - 0.8 \times 10^5$ cells in a 24-well plate in 0.45 ml of medium overnight will achieve the desired density of 30–50% confluency. If using culture plates of a different size, adjust the starting volume of X-tremeGENE siRNA Transfection Reagent and the starting mass of siRNA in proportion to the relative surface area (see table 1).

Ratio Overview

Preparation of a complex that is sufficient for a single well of a 24-well plate at three different concentrations.

Preparation of X-tremeGENE siRNA Transfection Reagent : siRNA Complex and Transfection of Cells in a 24-well plate

For initial optimization, use 10 : 2, 2.5 : 0.5, and 1 : 0.2 ratios of X-tremeGENE siRNA Transfection Reagent (μ l) to siRNA (μ g), respectively. The preparation of the complex for a single well of a 24-well plate is described below. These ratios will function very well for commonly used adherent cells.

⚠ The X-tremeGENE siRNA Transfection Reagent : siRNA complex must be prepared in medium that does not contain serum, even if the cells are transfected in the presence of serum.

🔗 For additional optimization tips, go to www.roche-applied-science.com/

1 Dilute X-tremeGENE siRNA Transfection Reagent with serumfree Opti-MEM® -1 medium (without antibiotics or fungicides):

⚠ Serum-free medium must be pipetted first. The order and manner of addition is critical.

- Label three small sterile tubes: “10,” “2.5,” and “1.” Pipet 40 μ l of serum-free medium into the first, 47.5 μ l into the second, and 49 μ l into the last tube.
- Pipet the X-tremeGENE siRNA Transfection Reagent directly into the medium without allowing contact with the walls of the plastic tube: 10 μ l X-treme GENE siRNA Transfection Reagent into the first tube, and 2.5 μ l of the reagent into the tube labeled “2.5” and 1 μ l of the reagent into the tube labeled “1”.

Tube label	SFM (serum-free medium)(μ l)	X-tremeGENE siRNA Transfection Reagent (μ l)
10	40	10
2.5	47.5	2.5
1	49	1

- Mix cautiously by pipetting up and down.

⚠ Do not vortex!

⚠ To avoid adversely affecting transfection efficiency, do not allow undiluted X-tremeGENE siRNA Transfection Reagent to come into contact with plastic surfaces (such as the walls of the tube containing the serum-free medium) other than pipette tips. Diluted siRNA should be combined with transfection reagent (step 3) within 5 min!

2 Dilute siRNA with serum-free Opti-MEM® -1 medium (without antibiotics or fungicides):

⚠ Serum-free medium must be pipetted first. The order and manner of addition is critical.

Label three small sterile tubes:

“50 + 2,” “50 + 0.5,” and “50 + 0.2.”

Pipet serum-free medium into all tubes to yield a final volume of 50 μ l.

Tube label	SFM (μ l) + siRNA (μ g)	Final volume (μ l)
50 + 2	50 + 2 (i.e. ~150 pmoles)	50
50 + 0.5	50 + 0.5 (i.e. ~40 pmoles)	50
50 + 0.2	50 + 0.2 (i.e. ~15 pmoles)	50

- Pipet the siRNA directly into the medium without allowing contact with the walls of the plastic tube: 2 μ g siRNA into the first tube, 0.5 μ g of the siRNA into the tube labeled “50 + 0.5” and 0.2 μ g of the siRNA into the tube labeled “50 + 0.2”.

- Mix cautiously by pipetting up and down.

⚠ Do not vortex!

⚠ To avoid adversely affecting transfection efficiency, do not allow undiluted siRNA to come into contact with plastic surfaces (such as the walls of the tube containing the serum-free medium) other than pipette tips.

Diluted siRNA and should be combined with transfection reagent (step 3) within 5 min!

3 Mix and incubate the complex:

- Mix the contents of the tube from Step 1 with that of Step 2 in the following order:

- Tube labeled „10“ with „50 + 2“
- Tube labeled „2.5“ with „50 + 0.5“
- Tube labeled „1“ with „50 + 0.2“

- Mix cautiously by pipetting up and down.

⚠ Do not vortex!

- Incubate the transfection reagent siRNA complex for 15 - 20 min at +15 to +25°C.

- Add the entire volume to each well of a 24 well plate.

4 Add complex to the cells:

- Remove culture vessel from the incubator.
- Removal of growth medium is not necessary.
- Add the transfection reagent : siRNA complex dropwise to the cells, and swirl the wells or flasks cautiously to ensure distribution over the entire plate surface.

5 Return the cells to the incubator until the assay for gene knock-down is performed. Once the X-tremeGENE siRNA Transfection Reagent : siRNA complex has been added to the cells, there is no need to remove and replace with fresh medium (as is necessary with some other transfection reagents).

- 🔗 The exposure of most common laboratory cell types (COS-7, NIH 3T3, HEK-293, HeLa) to the reagent: siRNA complex until measurement of the gene-knockdown (24–72 hours later) does not affect the results. When using serum-free medium during the transfection procedure (step 4), replace the medium with serum-containing medium 3–8 hours after transfection.

Optimizing Ratio of X-tremeGENE siRNA Transfection Reagent, siRNA and Plasmid DNA in the given Plate Formats

Refer to the table below (table 1) when setting up your transfection reactions in other plate formats. The starting volume and mass is based on a X-tremeGENE siRNA Transfection Reagent : siRNA ratio of 5 : 1. The ranges cover very different ratios. When varying the siRNA concentration, make sure to keep the X-tremeGENE siRNA Transfection Reagent in a ratio of 2-10 : 1 to the siRNA mass.

Culture plate diameter [mm]	96-well plate	24-well plate	6-well plate
Surface area cm ²	0.4	1.9	9
Plated cells [$\times 10^5$] suggested for start	0.05-0.2 0.1	0.1-0.8 0.4	1-4 2
Medium volume [ml]	0.15	0.45	2.0
siRNA [μ g] range suggested for start [μg]	0.05-0.3 0.15	0.1-1.0 0.5 (~40 pmoles)	0.4-5.0 2.0
Dilution volume [μ l]	in 15	in 50	in 100
X-tremeGENE siRNA Transfection Reagent [μ l] suggested for start [μl]	0.1-4.0 0.8	0.5-10 2.5	2-35 10
Dilution volume [μ l]	in 15	in 50	in 100
Total volume [ml]	0.18	0.55	2.2

Table 1

Co-Transfection Experiments

The X-tremeGENE siRNA Transfection Reagent has the potential for co-transfection of short inhibitory RNAs and plasmid DNA. Usually transfecting plasmids requires a higher cell density at the point of transfection compared to siRNA. Follow the suggestions when performing co-transfection experiments:

- Plate cells such that they reach 90% confluency at the time of transfection
- Maintain the same total reagent : total nucleic acid ratio as that used for siRNA alone in your system. (If you need to increase the total amount of nucleic acid, then increase the amount of transfection reagent in proportion to the total amount [μ g] of nucleic acid)
- Use the table below (table 2) for optimizing X-tremeGENE siRNA Transfection Reagent, siRNA and plasmid DNA in the given plate formats

⚠ Always use a volume of X-tremeGENE siRNA Transfection Reagent that is at least 3-fold in excess of the total final mass of nucleic acid.

Culture plate diameter [mm]	96-well plate	24-well plate	6-well plate
Surface area cm ²	0.4	1.9	9
Plated cells [$\times 10^5$] suggested for start	0.05 - 0.2 0.1	0.1 - 0.8 0.4	1 - 4 2
Medium volume [ml]	0.15	0.45	2.0
siRNA [μ g] range suggested for start [μg]	0.05 - 0.3 0.08	0.1 - 1.0 0.25	0.4 - 5.0 1.0
DNA [μ g]	0.15	0.5	2.0
Nucleic acid dilution volume	15	50	100
X-tremeGENE siRNA Transfection Reagent [μ l] suggested for start [μl]	0.1 - 4.0 0.8	0.5 - 10 2.5	2 - 35 10
Dilution volume [μ l]	in 15	in 50	in 100
Total volume [ml]	0.18	0.55	2.2

Table 2

3. Troubleshooting

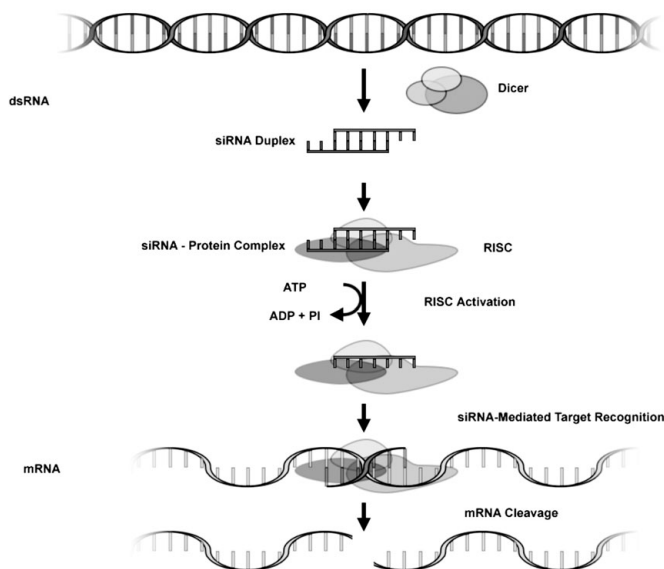
Observations	Possible Cause	Recommendation
Low/No knockdown levels observed	Low transfection efficiency	
	Cells were grown confluent at the time of transfection.	Seed cells at a lower density that they reach 30-50% confluency at the time of transfection.
	Cells passaged too often or used from stationary phase.	Use cells with low passage number and only cell cultures that were regularly passaged at log phase.
	Not enough siRNA used.	Increase the amount of siRNA.
	Not enough X-tremeGENE siRNA Transfection Reagent used.	Optimize transfection conditions for each cell line by varying the amount of siRNA: X-tremeGENE transfection complex and the ratio of siRNA : X-tremeGENE siRNA Transfection Reagent.
	Antibiotics added during transfection.	Avoid the addition of antibiotics during transfection.
	Serum present during siRNA : X-tremeGENE complex formation.	Use serum-free medium during complex formation.
	siRNA not active	
	Target sequence not suited.	Select another target region.
	d-siRNA degraded	Check integrity of siRNA on polyacrylamide or agarose gels. Do not store siRNA in water. Use a sterile RNase free buffer containing 10 mM Tris, pH 8.0, 20 mM NaCl, 1 mM EDTA for storage. Store siRNA aliquoted at -15 to -25°C and avoid repeated freeze/ thaw cycles.
Cytotoxic effects after transfection observed	Large cellular amounts or high stability of the targeted mRNA or protein	Perform a time course experiment and determine the time when the highest degree of knockdown is obtained. Perform qPCR analysis (e.g., by LightCycler) to measure mRNA levels when only low knock down on protein level is observed. Repeat the addition of siRNA: X-tremeGENE transfection complex and refresh medium for very long-lived target protein species.
	Too much X-tremeGENE siRNA Transfection Reagent used.	Reduce/optimize amounts of X-tremeGENE siRNA Transfection Reagent for each cell line.
	Cells are very sensitive to transfection	Remove transfection medium and add new prewarmed serum containing medium after 4-6 hours. (This will not reduce transfection efficiency.)
Non-specific off-target gene knockdown observed	Unpurified d-siRNA used for transfection	To avoid a general translation block and initiation of apoptosis, carefully purified siRNA of less than 30 nucleotides are necessary for most somatic mammalian cell lines (7,8).
	Target sequence siRNA sense or antisense strand contains strong homology to other genes.	Select a new target sequence. Lower the concentration of the siRNA Limit the size of the target sequence to <1.0 kb when using d-siRNA.

4. Additional Information on this Product

Background Information

The RNAi Pathway:

RNA interference (RNAi) is the process of sequence specific, post-transcriptional gene silencing in animals and plants, induced by double-stranded RNA (dsRNA) that triggers the degradation of complementary mRNA. In eukaryotic organisms *in vivo* or *in vitro* generated long double-stranded RNAs are cleaved into 21 -23 nucleotide long short interfering RNAs (siRNA) by a RNase III like enzyme activity called DICER (2). The siRNA is then taken up by the RNA-induced silencing complex (RISC), which anneals one of the siRNA strands to the complementary region of the mRNA and finally cleaves the mRNA (3).



References

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- Bernstein, E. et al (2001) *Nature* **409**, 363-366.
- Hammond, S.M. et al (2000) *Nature* **404**, 293-296.
- Bass, B.L. (2001) *Nature* **411**, 428-429.
- Hannon, G.J (2002) *Nature* **418**, 244-251.
- Kaufmann, R.J. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 11693-11695.
- Stark, G.R. et al (1998) *Annu. Rev. Biochem.* **67**, 227-264.

Quality Control

Activity assay: 1 - 2.5 microliters of X-tremeGENE siRNA Transfection Reagent is combined with 0.1 - 0.35 µg of siRNA against the HPRT housekeeping gene, and used to transfect Hek 293 cells (in a monolayer [30-50% confluent]) in the presence of 10% fetal bovine serum (FBS). Following transfection, the decrease of HPRT mRNA cells is analyzed with the LightCycler Instrument. Typically, a knockdown between 80% - 95 % is observed.

Cytotoxicity analysis: X-tremeGENE siRNA Transfection Reagent is routinely controlled for cytotoxicity with the WST-1, Cell Proliferation Reagent on Hek 293 cells, that are exposed to siRNA/ X-tremeGENE complexes for 72 hours in the presence of serum and without a change of the medium.

Absence of microbial contamination: The absence of microbial contamination is verified by a 1 week incubation of 25 µl of X-tremeGENE siRNA.

5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ① ②	Stages in a process that usually occur in the order listed
Numbered instructions labeled ① ②	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
ⓘ	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com

Product	Pack Size	Cat. No.
Transfection Reagents		
Cell Proliferation Reagent WST-1	2.500 tests	11 644 807 001
FuGENE 6 Transfection Reagent	1 ml 0.4 ml	11 814 443 001 11 815 091 001
DOTAP Liposomal Transfection Reagent	2 ml (5 x 0.4ml)	11 202 375 001
Protease Inhibitors		
Complete, EDTA-free	20 tablets	11 873 580 001
Protein Detection		
LumiLight ^{PLUS} Western Blotting Substrate	400 ml	12 015 196 001

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