For life science research only.
Not for use in diagnostic procedures.

X-tremeGENE siRNA Transfection Reagent

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For the transfection of siRNA into animal cells

Cat. No. 04 476 093 001	1 ml 400 transfections in 24-well plates or 1,600 transfections in 96-well plates
Cat. No. 04 476 115 001	5 x 1 ml 2,000 transfections in 24-well plates or 8,000 transfections in 96-well plates

Store the reagent at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	colorless	X-tremeGENE siRNA Transfection Reagent	▪ Proprietary blend of lipids and other components supplied in aqueous solution. ▪ Filtered through 0.2 µm pore-size membrane, and packaged in polypropylene tubes. ▪ Does not contain any ingredients of human or animal origin.	04 476 093 001	1 vial, 1 ml
				04 476 115 001	5 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The reagent is shipped at +15 to +25°C.
When stored at +2 to +8°C, the reagent is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	colorless	X-tremeGENE siRNA Transfection Reagent	Store at +2 to +8°C. The reagent is stabilized for extended storage. ⚠ ***Shipping temperature of this product is different from the storage temperature. This will not affect product performance or product stability.*** ⚠ ***Do not freeze.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard cell culture equipment, such as biohazard hoods and incubators
- Sterile pipettes, polypropylene tubes, and culture plates
- Vortex mixer

For dilution of siRNA and X-tremeGENE siRNA Transfection Reagent

- Prewarmed serum-free Opti-MEM I Medium or serum-free medium, without additives or supplements
- siRNA solution between 0.2 µg/µl (15 pmoles/µl [15 µM]) and 2 µg/µl (150 pmoles/µl [150 µM]) in sterile buffer

Growing cells

- Mammalian cell line of interest
- Select subconfluent cultures in log phase for preparation of cell cultures
- Quantify cell number to reproducibly plate the same number of cells

1.4. Application

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

- High transfection efficiency in many common cell types, including HeLa, NIH 3T3, HEK-293, PC-3, and COS-7.
- A single reagent for siRNA- and co-transfection-based RNA experiments.
- Low cytotoxicity, and the media change 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

General Considerations

siRNA purity and fluorescently labeled siRNAs

- Determine the siRNA purity using a OD 260/280 nm ratio. The ratio should be ≥1.9.
- When using siRNA preparations from *in vitro* transcription reactions, a size of <30 base pairs 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 (Kim D, et al, 2004). This publication describes that the 5′ triphosphate 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 intra- and inter-experimental variance in transfection efficiency using cells that are regularly passaged, proliferating well in a log-growth phase, and plated at a consistent density.
- For best results, accurately quantify cell concentration using a hemocytometer or automated system.
- Cells must be healthy and free of mycoplasma.
- Cells should have a low passage number to achieve best results.

Other media additives

- In some cell types, antimicrobial agents, such as antibiotics and fungicides commonly included in cell culture media may adversely affect the transfection efficiency of X-tremeGENE siRNA Transfection Reagent. If possible, exclude additives in initial experiments. Once high-efficiency conditions have been established, these components can be added back while monitoring transfection results.
- 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.
  ℹ *Any medium can be used for cell cultivation and during transfection, however, use Opti-MEM I Medium for dilution of the siRNA and the X-tremeGENE siRNA Transfection reagent, see section,* **Preparation of X-tremeGENE siRNA Transfection Reagent:siRNA complex and transfection of cells in a 24-well plate**.
- 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 to 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, always determine the transfection efficiency when using a new cell line. The knockdown of the endogenous human HPRT housekeeping gene can be measured with qPCR. 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 to 72 hours. The length of incubation depends upon the siRNA, cell type being transfected, 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.

Number of tests

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.

2.2. Protocols

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 to 0.8 × 10^5 cells in a 24-well plate in 0.45 ml of medium overnight will achieve the desired density of 30 to 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 in section, **Optimizing ratio of X-tremeGENE siRNA Transfection Reagent, siRNA, and plasmid DNA in various plate formats**.

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

The preparation of the complex for a single well of a 24-well plate is described in the following steps.

⚠ ***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.***

❶ Dilute X-tremeGENE siRNA Transfection Reagent with serum-free Opti-MEM I Medium.

i The medium must not contain antibiotics or fungicides.

⚠ ***Pipette serum-free medium first; the order and manner of addition is critical.***

– Label three small sterile tubes: 10, 2.5, and 1, and pipette 40 µl of serum-free medium into the first, 47.5 µl into the second, and 49 µl into the last tube.
– Pipette the X-tremeGENE siRNA Transfection Reagent directly into the medium avoiding contact with the walls of the plastic tube: 10 µl X-tremeGENE siRNA Transfection Reagent into the first tube, 2.5 µl of the reagent into the tube labeled 2.5, and 1 µl of the reagent into the tube labeled 1.

Tube Label	Serum-Free Media [µl]	X-tremeGENE siRNA Transfection Reagent [µl]
10	40	10
2.5	47.5	2.5
1	49	1.0

– Mix carefully 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; pipette tips are ok.

⚠ ***Combine diluted siRNA with transfection reagent (Step 3) within 5 minutes.***

❷ Dilute siRNA with serum-free Opti-MEM I Medium.

i *The medium must not contain antibiotics or fungicides.*

⚠ ***Pipette serum-free medium first; the order and manner of addition is critical.***

– Label three small sterile tubes: 50 + 2, 50 + 0.5, and 50 + 0.2.
– Pipette serum-free medium into all tubes to yield a final volume of 50 µl.

Tube Label	Serum-Free Media [µl] + siRNA [µg]	Final Volume [µl]
50 + 2	50 + 2 (≈ 150 pmoles)	50
50 + 0.5	50 + 0.5 (≈ 40 pmoles)	50
50 + 0.2	50 + 0.2 (≈ 15 pmoles)	50

– Pipette the siRNA directly into the medium avoiding contact with the walls of the plastic tube: 2 µg siRNA into the first tube, 0.5 µg siRNA into the tube labeled 50 + 0.5, and 0.2 µg siRNA into the tube labeled 50 + 0.2.
– Mix carefully 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; pipette tips are ok.

⚠ ***Combine diluted siRNA with transfection reagent (Step 3) within 5 minutes.***

❸ 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 carefully by pipetting up and down.

⚠ ***Do not vortex.***

– Incubate the transfection reagent:siRNA complex for 15 to 20 minutes at +15 to +25°C.
– Add the entire volume to each well of a 24-well plate.

❹ 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/flask surface.

❺ Return the cells to the incubator until the assay for gene knockdown 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 other transfection reagents.

i *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 to 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 to 8 hours after transfection.*

Optimizing ratio of X-tremeGENE siRNA Transfection Reagent, siRNA, and plasmid DNA in various plate formats

Refer to the following table when setting up your transfection reactions in other plate formats. The starting volume and mass is based on an X-tremeGENE siRNA Transfection Reagent:siRNA ratio of 5:1. The ranges cover multiple ratios. When varying the siRNA concentration, keep the X-tremeGENE siRNA Transfection Reagent in a ratio of 2 to 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	9.0
Plated cells [$\times 10^5$] range	0.05 – 0.2	0.1 – 0.8	1 – 4
Suggested starting point	**0.1**	**0.4**	**2.0**
Medium volume [ml]	0.15	0.45	2.0
siRNA [µg] range	0.05 – 0.3	0.1 – 1.0	0.4 – 5.0
Suggested starting point [µg]	**0.15**	**0.5 (≈ 40 pmoles)**	**2.0**
Dilution volume [µl]	15	50	100
X-tremeGENE siRNA Transfection Reagent [µl] range	0.1 – 4.0	0.5 – 10	2.0 – 35
Suggested starting point [µl]	**0.8**	**2.5**	**10**
Dilution volume [µl]	15	50	100
Total Volume [ml]	0.18	0.55	2.2

Co-transfection experiments

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. 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, increase the amount of transfection reagent in proportion to the total amount of nucleic acid (µg).

Refer to the following table when optimizing X-tremeGENE siRNA Transfection Reagent, siRNA, and plasmid DNA in the various 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	9.0
Plated cells [$\times 10^5$] range	0.05 – 0.2	0.1 – 0.8	1 – 4
Suggested starting point	**0.1**	**0.4**	**2.0**
Medium volume [ml]	0.15	0.45	2.0
siRNA [µg] range	0.05 – 0.3	0.1 – 1.0	0.4 – 5.0
Suggested sarting point [µg]	**0.08**	**0.25**	**1.0**
DNA [µg]	0.15	0.5	2.0
Dilution volume [µl]	15	50	100
X-tremeGENE siRNA Transfection Reagent [µl] range	0.1 – 4.0	0.5 – 10	2.0 – 35
Suggested starting point [µl]	**0.8**	**2.5**	**10**
Dilution volume [µl]	15	50	100
Total Volume [ml]	**0.18**	**0.55**	**2.2**

2.3. Parameters

Working Concentration

Required amount of X-tremeGENE siRNA Transfection Reagent

To optimize, use 10:2, 2.5:0.5, and 1:0.2 ratios of microliter (µl) X-tremeGENE siRNA Transfection Reagent to microgram (µg) siRNA. These ratios will function very well for commonly used adherent cells.

3. Troubleshooting

Observation	Possible cause	Recommendation
Low or no knockdown levels observed.	Low transfection efficiency.	
	Cells were grown confluent at the time of transfection.	Seed cells at a lower density so they reach 30 to 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 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.	Do not add antibiotics during transfection.
	Serum present during siRNA: X-tremeGENE complex formation.	Use serum-free medium during complex formation.
	siRNA not active.	
	Target sequence not suitable.	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.
	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., using the LightCycler® Instrument) to measure mRNA levels when only low knockdown on protein level is observed.
		Repeat the addition of siRNA:X-tremeGENE transfection complex and refresh medium for every long-lived target protein species.
Cytotoxic effects after transfection observed.	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 to 6 hours. This will not reduce transfection efficiency.
	Unpurified d-siRNA used for transfection.	To avoid a general translation block and initiation of apoptosis, carefully purified siRNA of less than 30 nucleotides is necessary for most somatic mammalian cell lines.
Nonspecific off-target gene knockdown observed.	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

4.1. Test Principle

RNAi mechanism

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 to 23 nucleotide long, short interfering RNAs (siRNA) by a RNAse III-like enzyme activity called DICER. 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 (Fig. 1).

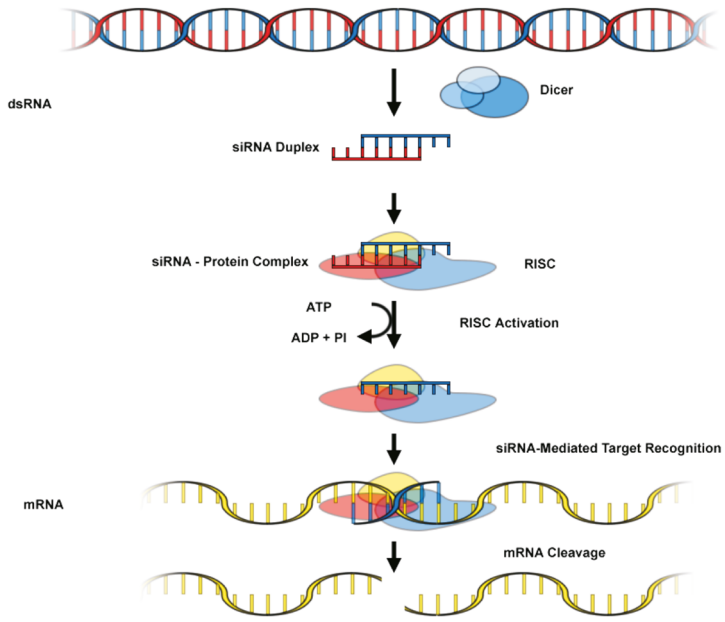

Fig. 1: RNAi **mechanism**

4.2. References

- Kim D, Longo M, Han Y, Lundberg P, Cantin E, Rossi JJ. Interferon induction by siRNAs and ssRNAs synthesized by phage polymerase. Nature Biotechnology. 2004;22:321-325.

4.3. Quality Control

Each lot of X-tremeGENE siRNA Transfection Reagent is tested using established quality control procedures.

Functional analysis

Cells are transfected using three different transfection reagent to NA ratios.

Absence of microbial contamination

The absence of microbial contamination is verified by a one-week incubation of 25 µl of X-tremeGENE siRNA Transfection Reagent.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update of the quality control section.

5.3. Trademarks

X-TREMEGENE is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

5.4. License Disclaimer

For patent license limitations for individual products please refer to: **List of biochemical reagent products**

5.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.6. Safety Data Sheet (SDS)

Please follow the instructions in the Safety Data Sheet (SDS).

5.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country. Country-specific contact information will be displayed.

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