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

X-tremeGENE 9 DNA Transfection Reagent

Version: 10
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For transient and stable transfection of eukaryotic cells

Cat. No. 06 365 779 001	0.4 ml
Cat. No. 06 365 787 001	1.0 ml
Cat. No. 06 365 809 001	5 x 1.0 ml

Store the reagent at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	white	X-tremeGENE 9 DNA Transfection Reagent	▪ Proprietary blend of lipids and other components supplied in 80% ethanol. ▪ Filtered through 0.2 µm pore-size membrane, and packaged in glass vials.	06 365 779 001	1 vial, 0.4 ml
				06 365 787 001	1 vial, 1 ml
				06 365 809 001	5 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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	white	X-tremeGENE 9 DNA Transfection Reagent	Store at +2 to +8°C. ⚠ ***The reagent remains fully functional even after repeated opening of the vial (at least five times over a two-month period), as long as the vial is tightly capped and stored at +2 to +8°C. Do not freeze.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard cell culture equipment, such as biohazard hoods and incubators
- Standard pipettes and micropipettes
- Vortex mixer

For plasmid preparation

- Purified plasmid stock (0.1 to 2.0 µg/µl) in sterile TE (10 mM Tris, 1 mM EDTA, pH 8.0) buffer or sterile water
- Genopure Plasmid Midi Kit* or Genopure Plasmid Maxi Kit*

For verification of vector function

- Assay appropriate for transfected gene
- G-418 Solution* or Hygromycin B* (optional for stable transfection experiments)

For transfection-complex formation

- Opti-MEM I Reduced Serum Medium or serum-free medium
- Sterile polypropylene tubes or round-bottom, 96-well plates

Growing cells

- 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 9 DNA Transfection Reagent is a non-liposomal multi-component reagent for experiments in cellular analysis. Its low cytotoxicity, minimal need for optimization, and ability to provide high transfection efficiency in a wide range of commonly used cell lines, even in the presence of serum, are especially suited for applications in cellular and molecular research.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Precautions

- Close the vial tightly with the lid immediately after removing the required amount of reagent from the vial.
- Always equilibrate the vial to +15 to +25°C; then vortex the X-tremeGENE 9 DNA Transfection Reagent 1 second prior to removing the required amount.
- Do not aliquot X-tremeGENE 9 DNA Transfection Reagent; store in the original glass vials.
- Minimize the contact of undiluted X-tremeGENE 9 DNA Transfection Reagent with plastic surfaces.
- The minimum amount of X-tremeGENE 9 DNA Transfection Reagent:DNA complex to use is 100 µl. Complex formation at lower volumes can significantly decrease transfection efficiency.
- Do not use tubes or microplates made of polystyrene when preparing the X-tremeGENE 9 Transfection Reagent:DNA complex. If only polystyrene materials are available, pipette the transfection reagent directly into serum-free medium, such as Opti-Mem.
- Do not use siliconized pipette tips or tubes.

Plasmid DNA

- For best results, accurately determine the plasmid DNA concentration using 260-nm absorption; do not estimate DNA by measuring gel band density. Determine DNA purity using a 260 nm/280 nm ratio; the optimal ratio is 1.8.
- Prepare the plasmid DNA solution using sterile TE (Tris/EDTA) buffer or sterile water at a concentration of 0.1 to 2.0 µg/µl.
- Use high quality DNA preparation kits to obtain endotoxin-free DNA.

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 9 DNA 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. Cell growth and/or transfection efficiency may be affected by variations in serum quality and medium formulations.

Verification of vector function

Optimize transfection conditions using a known positive-control reporter gene construct before transfecting cells with a new vector construct:

- Determine transfection efficiency using a reporter gene assay, such as β-Gal*, Luciferase*, or SEAP*.
- Sequence flanking vector insert regions to verify the integrity of your new construct.

Number of tests

Using the standard procedure, 1 ml of X-tremeGENE 9 DNA Transfection Reagent can be used to perform up to 6,600 transfections in 96-well plates using the 3:1 ratio and up to 10,000 transfections using a 2:1 ratio.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Preparation of cells for transfection

Adherent cells: Plate cells approximately 24 hours before transfection making sure cells are at the optimal concentration in the appropriate cell culture vessel.
Suspension cells: Plate freshly passaged cells at optimal concentration.

Transfection protocol

1. Allow X-tremeGENE 9 DNA Transfection Reagent, DNA, and diluent to equilibrate to +15 to +25°C.
   – Briefly vortex the X-tremeGENE 9 DNA Transfection Reagent vial.

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2. Dilute X-tremeGENE 9 DNA Transfection Reagent with serum-free medium, such as Opti-MEM to a concentration of 3 µl reagent/100 µl medium for a ratio of 3:1, and 6 µl reagent/100 µl medium for a ratio of 6:1, using a sterile tube and gentle mixing.

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3. Add 1 µg of DNA to 100 µl of diluted X-tremeGENE 9 DNA Transfection Reagent; mix gently.
   ⚠ ***Use a minimum of 100 µl of diluent. Lower volumes may significantly decrease transfection efficiency.***
   ℹ *Use sterile tubes or tissue culture-treated, round-bottom, 96-well plates to produce the complex.*
   ⚠ ***To avoid adversely affecting transfection efficiency, do not allow undiluted X-tremeGENE 9 DNA Transfection Reagent to come into contact with plastic surfaces. Do not use siliconized pipette tips or tubes.***

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4. Incubate the transfection reagent:DNA complex for 15 minutes at +15 to +25°C.
   ℹ *Some ratios and cell types may require longer incubation times, up to 30 minutes. Determine the times for your particular cell line and the ratio used.*

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5. Remove the culture vessel from the incubator; removal of growth medium is not necessary.
   – Add the transfection complex to the cells in a dropwise manner.
   ℹ *See section,* **Guidelines for preparing Reagent:DNA complex for various culture vessel sizes** *to determine component amounts corresponding to the surface area of the cell culture vessel used.*
   – Gently shake or swirl the wells or flasks to ensure even distribution over the entire plate surface. If available, use a rotating platform shaker for 30 seconds at low speed for mixing 96-well plates.
   – Once the transfection reagent:DNA complex has been added to the cells, there is no need to replace with fresh medium as may be required with other transfection reagents.

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6. Following transfection, incubate cells for 18 to 72 hours before measuring protein expression.
   – The duration of incubation will depend on many factors, including the transfected vector construct, the cell type being transfected, the cell medium, cell density, and the type of protein being expressed.
   – After the incubation period, measure protein expression using an assay appropriate for your system.

Guidelines for preparing reagent:DNA complex for various culture vessel sizes

Culture vessel	Surface area [cm²]	Total volume of medium [ml]	Suggested amount of 100 µl transfection complex to add to each well [µl]	DNA using 3:1 or 6:1 ratio [µg]	Final amount of X-tremeGENE 9 DNA Transfection Reagent using 3:1 ratio [µl]	Final amount of X-tremeGENE 9 DNA Transfection Reagent using 6:1 ratio [µl]
96-well plate (1 well)	0.3	0.1	5	0.05	0.15	0.3
48-well plate (1 well)	1.0	0.3	15	0.15	0.45	0.9
24-well plate (1 well)	1.9	0.5	25	0.25	0.75	1.5
12-well plate (1 well)	3.8	1.0	50	0.5	1.5	3.0
35-mm dish	8.0	2.0	100	1.0	3.0	6.0
6-well plate (1 well)	9.4	2.0	100	1.0	3.0	6.0
60-mm dish	21	5.0	250	2.5	7.5	15
10-cm dish	55	10	500	5.0	15	30
T-25 flask	25	6.0	300	3.0	9.0	18
T-75 flask	75	20	1,000	10	30	60

Additional information

- As with any experiment, include appropriate controls. Prepare culture wells with cells that remain untransfected, cells with transfection reagent alone, and cells with DNA alone.
- For stable transfection experiments, do not change the complex-containing medium until the cells are passaged. At that time, include appropriate selection antibiotics, such as G-418 Solution or Hygromycin B.
- To prepare transfection complexes for different-sized containers or parallel experiments, adjust component amounts corresponding to the surface area of the cell culture vessel used, see section, **Guidelines for preparing Reagent:DNA complex for various culture vessel sizes**.
- For ease-of-use, when transfecting small volumes into 96-well plates containing 0.1 ml culture medium per well, prepare 100 µl of transfection complex, and then add 5 to 10 µl to each well, depending on cell type.
- The optimal ratio of transfection reagent to DNA, and the optimal total amount of complex will depend on the cell line, cell density, day of assay, and gene expressed.
- After performing the optimization experiment in which several different ratios are tested, select a ratio in the middle of the plateau optimum for future experiments.

2.3. Parameters

Working Concentration

Required amount of X-tremeGENE 9 DNA Transfection Reagent

To optimize, first transfect a monolayer of cells that is 70 to 90% confluent, using 3:1, 6:1, and 6:2 ratios of microliter (µl) X-tremeGENE 9 DNA Transfection Reagent to microgram (µg) DNA. A ratio of 3:1 of microliter (µl) X-tremeGENE 9 DNA Transfection Reagent to microgram (µg) DNA has been shown to be optimal for many cell types.

Lower cell confluencies have also been tested successfully.

The recommended starting concentration is 3:1. For most cell types, these X-tremeGENE 9 DNA Transfection Reagent to DNA ratios provide excellent transfection efficiency.

Further optimization using rations from 2:1 to 7:1 may increase transfection efficiency in your particular application. In addition to varying the ratio, other parameters may also be evaluated, such as the amount of transfection complex added. For additional optimization guidelines, see section, **Troubleshooting.**

3. Results

CHO-K1 cells were transfected with a GFP encoding pcDNA3.1 plasmid containing a CMV promoter with two different transfection reagents (Figures 1 and 2). CHO-K1 cells were observed under fluorescence and bright field microscopy at 10× magnification.

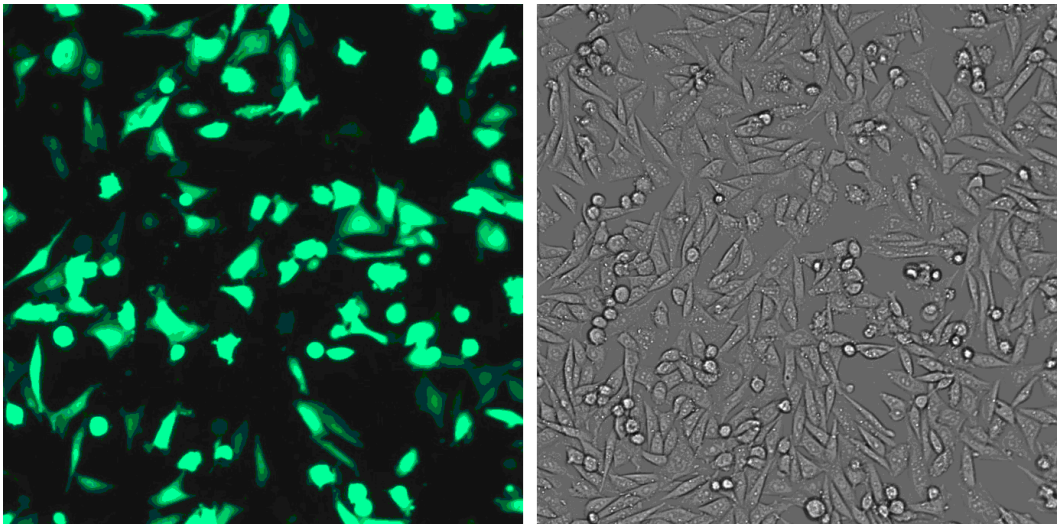

Fig. 1: X-tremeGENE 9 DNA Transfection Reagent (3:1 ratio).

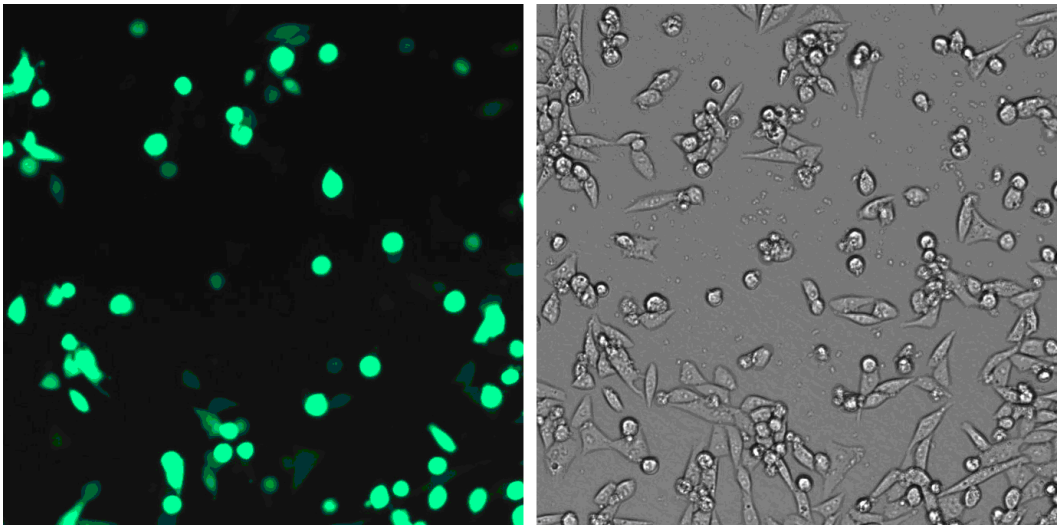

Fig. 2: Competitor transfection reagent (2:1 ratio).

4. Troubleshooting

Low transfection efficiency.	Suboptimal X-tremeGENE 9 DNA Transfection Reagent:DNA ratio.	Titrate optimal X-tremeGENE 9 DNA Transfection Reagent:DNA ratio, see section, **General Considerations, Required amount of X-tremeGENE 9 DNA Transfection Reagent**.
	Insufficient number of cells.	Determine optimal cell density for each cell type. For most cell types, 70 to 90% confluency at transfection is optimal.
	X-tremeGENE 9 DNA Transfection Reagent:DNA complexes did not form well.	Prepare complexes in serum-free medium, such as Opti-MEM.
		Do not use siliconized pipette tips or tubes.
		Do not aliquot the X-tremeGENE 9 DNA Transfection Reagent.
	Incubation time of transfection not optimal.	Determine the optimal incubation time (18 to 72 hours); optimal for most cell types and plasmids is 24 to 48 hours.
	Inhibition by media components.	Some media components, such as polyanions may influence the transfection.
	Suboptimal X-tremeGENE 9 DNA Transfection Reagent:DNA complex.	Titrate optimal X-tremeGENE 9 DNA Transfection Reagent:DNA ratio, see section, **General Considerations, Required amount of X-tremeGENE 9 DNA Transfection Reagent**.
High cytotoxicity	Cell density not optimal.	Determine optimal cell density for each cell type. For most cell types, 70 to 90% confluency at transfection is optimal; other confluencies may increase cell viability.
	Cells are cultured in serum-free medium.	Transfection using X-tremeGENE 9 DNA Transfection Reagent in cells cultured in serum-free medium is possible, however, toxicity may be higher when serum is absent.
	X-tremeGENE 9 DNA Transfection Reagent:DNA complexes and cells not mixed well.	Add X-tremeGENE 9 DNA Transfection Reagent dropwise to the cells, then gently rock the dish/plate back and forth and from side to side to evenly distribute the complexes.
	Plasmid preparation contaminated with endotoxin.	Use highly purified, contaminant-free DNA for transfection.
	Transfected protein is cytotoxic or is produced at high levels.	Reduced viability or slow growth rates may be due to high levels of protein expression, with cellular metabolism directed toward production of the heterologous protein. *The expressed protein may also be cytotoxic at the expressed levels.*
	Too much transfection complex for number of cells.	Increase the number of plated cells, and/or decrease the total amount of complex added to the cells.

5. Additional Information on this Product

5.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

Functional analysis

Cells are transfected with a reporter gene vector DNA using X-tremeGENE 9 DNA Transfection Reagent (ratio 3:1 µl/µg DNA). Reporter gene activity is monitored by chemiluminescent detection. Using a standard curve analysis method, total amounts of recombinant protein per well are measured to ensure levels that are within specification.

Viability analysis

Cell viability is assessed using the Cell Viability Imaging Kit*. The same transfection reagent to DNA ratio is used for both the functional and viability analysis.

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Hygromycin B	1 g, 20 ml	10 843 555 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit, 500 assays (microplate format) 250 assays (tube format)	11 758 241 001
G-418 Solution	20 ml, 1 g	04 727 878 001
	100 ml, 5 x 20 ml	04 727 894 001
Genopure Plasmid Midi Kit	1 kit, 20 preparations	03 143 414 001
Genopure Plasmid Maxi Kit	1 kit, 10 preparations	03 143 422 001
Cell Viability Imaging Kit	1 kit, 5 × 96 reactions	06 432 379 001

6.4. Trademarks

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

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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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