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Roche

X-tremeGENE HP DNA Transfection Reagent

For transient and stable transfection of eukaryotic cells

Version 08
Content version: January 2014

Cat. No. 06 365 752 001	Trial-pack
Cat. No. 06 366 244 001	0.4 ml
Cat. No. 06 366 236 001	1 ml
Cat. No. 06 366 546 001	5 × 1 ml

Store at –15 to –25°C

1. What this Product Does

Number of Tests

Using the standard procedure, 1 ml of X-tremeGENE HP DNA Transfection Reagent can be used to perform up to 10,000 transfections in 96-well plates.

Formulation

X-tremeGENE HP DNA Transfection Reagent is a proprietary blend of lipids and other components supplied in 80% ethanol, filtered through 0.2 µm pore size membrane, and packaged in glass vials. It does not contain any ingredients of human or animal origin.

Storage and Stability

Store X-tremeGENE HP DNA Transfection Reagent at –15 to –25°C, with the lid tightly closed. The reagent is stable until the expiration date printed on the label when stored under these conditions.

- X-tremeGENE HP DNA Transfection 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 recapped and stored at -15 to -25°C.
- Note that the shipping temperature of this product is different from the storage temperature. These different temperatures will not affect product performance or product stability.

Special Handling

- ⚠ After removing the amount required, tightly close the vial with the lid immediately after use.
- ⚠ Always bring the vial to +15 to +25°C and mix X-tremeGENE HP DNA Transfection Reagent prior to removing the amount required vortexing for one second.
- ⚠ Do not aliquot X-tremeGENE HP DNA Transfection Reagent; store in the original glass vials.
- ⚠ Minimize the contact of undiluted X-tremeGENE HP DNA Transfection Reagent with plastic surfaces.
- ⚠ For use, the minimum amount of X-tremeGENE HP DNA Transfection Reagent: DNA complex is 100 µl. Complex formation at lower volumes can significantly decrease transfection efficiency.
- ⚠ Do not use tubes or microplates made of polystyrene for X-tremeGENE HP Transfection Reagent : DNA complex preparation. When not able to avoid polystyrene materials, make certain to pipet the transfection reagent directly into the serum-free medium (*e.g.,* Opti-Mem).
- ⚠ Do not use siliconized pipette tips or tubes.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform transfection assays using X-tremeGENE HP DNA Transfection Reagent include:

➤ **Standard Laboratory Equipment**.
- Standard cell culture equipment (*e.g.,* biohazard hoods, incubators)
- Standard pipettes and micropipettes
- Vortex mixer

➤ **For Plasmid Preparation**
- Purified plasmid stock (0.1 – 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* to prepare plasmid

➤ **For Verification of Vector Function**
- Assay appropriately 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

Application

X-tremeGENE HP DNA Transfection Reagent is a high performance transfection reagent, free of animal-derived components. Benefits of X-tremeGENE HP DNA Transfection Reagent include:

- Designed to transfect a broad range of eukaryotic cells, including insect cells, many cell lines not transfected well by other reagents, and hard-to-transfect cell lines (*e.g.,* HT-1080, K-562, HepG2).
- Can be successfully used in a variety of applications, such as gene expression analysis and protein production using transiently transfected cells, generation of stable cell lines, expression of shRNA for gene knockdown studies, drug discovery programs, and target evaluation. Samples and detailed transfection protocols are available at ***http://www.powerful-transfection.com.***
- Produces minimal cytotoxicity or changes in morphology when adequate numbers of cells are transfected, eliminating the requirement to change media after adding the transfection complex.
- Suitable for transient and stable transfection.
- Functions very well in the presence or absence of serum.

2. How to Use this Product

2.1 Before You Begin

Required Amount of X-tremeGENE HP DNA Transfection Reagent

To optimize, first transfect a monolayer of cells that is 70 – 90% confluent, using 1:1, 2:1, 3:1 and 4:1 ratios of microliter (µl) X-tremeGENE HP DNA Transfection Reagent to microgram (µg) DNA. A ratio of 3:1 of microliter (µl) X-tremeGENE HP 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 a 3:1. For most cell types, these X-tremeGENE HP DNA Transfection Reagent to DNA ratios provide excellent transfection efficiency.

Further optimization 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 3, Troubleshooting and visit ***http://www.powerful-transfection.com.***

Plasmid DNA

- For best results, accurately determine the plasmid DNA concentration using 260-nm absorption; estimates of DNA by measuring gel band density are not recommended. 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 hematocytometer 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 (*e.g.,* antibiotics and fungicides) commonly included in cell-culture media may adversely affect the transfection efficiency of X-tremeGENE HP 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.

2.2 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.

2.3 Transfection Procedure

❶	Allow X-tremeGENE HP DNA Transfection Reagent, DNA and diluent to equilibrate to +15 to +25°C. Briefly vortex the X-tremeGENE HP DNA Transfection Reagent vial.
❷	Dilute DNA with appropriate diluent (*e.g.,* serum-free medium) to a final concentration of 1 µg plasmid DNA /100 µl medium (0.01 µg/µl). Mix gently.
❸	Place 100 µl of diluent, containing 1 µg DNA into each of four sterile tubes labeled 1:1, 2:1, 3:1, and 4:1. ⚠ Use a minimum of 100 µl of diluent. Lower volumes may significantly decrease transfection efficiency. Use sterile tubes or tissue culture treated round-bottom, 96-well plates to produce the complex.
❹	Pipet the X-tremeGENE HP DNA Transfection Reagent (1, 2, 3, or 4 µl) directly into the medium containing the diluted DNA without coming into contact with the walls of the plastic tubes. Mix gently. ⚠ To avoid adversely affecting transfection efficiency, do not allow undiluted X-tremeGENE HP DNA Transfection Reagent to come into contact with plastic surfaces. Do not use siliconized pipette tips or tubes.
❺	Incubate the transfection reagent:DNA complex for 15 minutes at +15 to +25°C. Some ratios and cell types may required longer incubation (up to 30 min). Determine this for your particular cell line and the ratio used.
❻	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. See Table 1 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 necessary with other transfection reagents)
❼	Following transfection, incubate cells for 18 – 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.

Notes:

- 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, the complex-containing medium should be left unchanged until the cells are passaged. At that time, include appropriate selection antibiotics (*e.g.,* 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 Table 1).
- 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 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.

Tab. 1: Guidelines for Preparing X-tremeGENE HP DNA Transfection Reagent: DNA Complex for Various Culture Vessel Sizes

Culture vessel	Surface Area (cm^2)	Total volume of medium (ml)	Suggested amount of 100 µl transfection complex to add to each well (µl)	DNA (µg) using 1:1 or 4:1 Ratio	Final amount of X-tremeGENE HP DNA Transfection Reagent (µl) using 1:1 Ratio	Final amount of X-tremeGENE HP DNA Transfection Reagent (µl) using 4:1 Ratio
96-well plate (1 well)	0.3	0.1	10	0.1	0.1	0.4
48-well plate (1 well)	1.0	0.3	30	0.3	0.3	1.2
24-well plate (1 well)	1.9	0.5	50	0.5	0.5	2
12-well plate (1 well)	3.8	1	100	1	1	4
35-mm dish	8	2	200	2	2	8
6-well plate (1 well)	9.4	2	200	2	2	8
60-mm dish	21	5	500	5	5	20
10-cm dish	55	10	1000	10	10	40
T-25 flask	25	6	600	6	6	24
T-75 flask	75	20	2000	20	20	80

2.4 Troubleshooting

Observation	Possible Cause	Recommendation
Low Transfection Efficiency	Suboptimal X-tremeGENE HP DNA Transfection Reagent : DNA ratio	Titrate optimal X-tremeGENE HP DNA Transfection Reagent : DNA ratio. Refer to the text in Section 2.1 “Before you begin”.
	Insufficient number of cells	Determine optimal cell density for each cell type. For most cell types, 70 – 90% confluence at transfection is optimal.
	X-tremeGENE HP DNA Transfection Reagent : DNA complexes did not form well	Prepare complexes in serum-free medium (*e.g.,* Opti-MEM). Do not use siliconized pipet tips or tubes. Do not aliquot the X-tremeGENE HP DNA Transfection Reagent.
	Incubation time of transfection	Determine the optimal incubation time (18 - 72 h). Optimal for most cell types and plasmids is 24 – 48h.
	Inhibition by media components	Some media components (*e.g.,* polyanions) may influence the transfection.
	Low volume of X-tremeGENE HP DNA Transfection Reagent : DNA complex	The minimum amount of X-tremeGENE HP DNA Transfection Reagent to DNA complex is 100 µl. Complex formation at lower volumes may significantly decrease the transfection efficiency; refer to the text in Section 1, “Special Handling”.
High Cytotoxicity	Cell density not optimal	For each cell type, the optimal density should be determined. For most cell types, 70 - 90 % confluence at transfection is recommended, but other confluencies may increase cell viability.
	Cells are cultured in serum-free medium	Transfection using X-tremeGENE HP DNA Transfection Reagent in cells cultured in serum-free medium is possible, however, toxicity may be higher when serum is absent.
	X-tremeGENE HP DNA Transfection Reagent : DNA complexes and cells not mixed well	Add X-tremeGENE HP DNA Transfection Reagent dropwise to the cells. 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. Note that 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.

3. Additional Information on This Product

Quality Control

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

Functional Analysis

Cells are transfected with a reporter gene vector DNA using X-tremeGENE HP 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.

4. Results

CHO-K1 cells were transfected with a GFP encoding pcDNA3.1 plasmid containing a CMV promoter with two different transfection reagents. CHO-K1 cells were observed under fluorescence and bright field microscopy at 10× magnification. Pictures were obtained using the Cellavista System 24 hours after transfection.

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

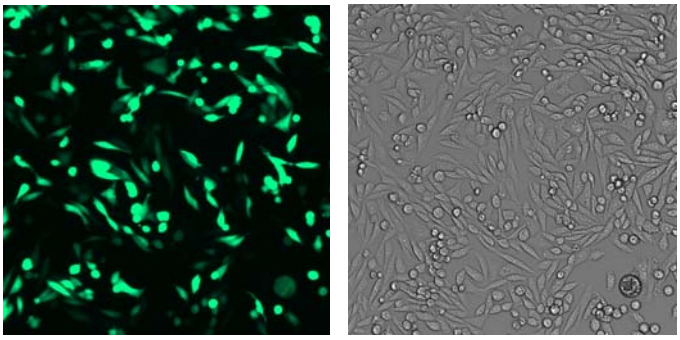
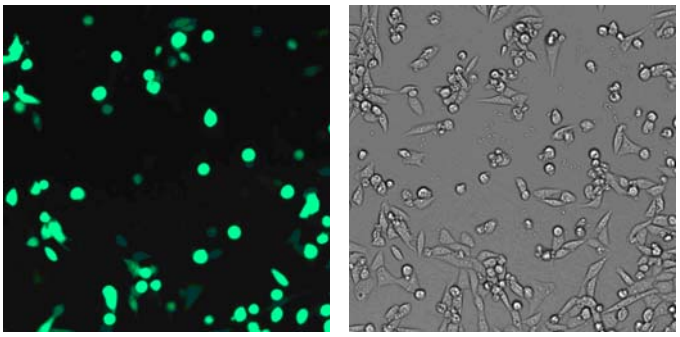


Fig. 2: Competitor transfection reagent (2:1 ratio)



5. Supplementary Information

Conventions

In this document, 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.

Text Conventions

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

Text Convention	Use
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Changes to Previous Version

New quality control test for viability analysis.

Ordering Information

Roche Applied Science provides a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Site on www.powerful-transfection.com

Product	Pack Size	Cat. No.
Apoptosis and Cell Death Products		
Cell Proliferation Reagent WST-1	25 ml (2,500 tests) 8 ml (800 tests)	11 644 807 001 05 015 944 001
Cytotoxicity Detection Kit^{PLUS} (LDH)	1 kit 400 tests in 96 wells 1 kit 2,000 tests in 96 wells	04 744 926 001 04 744 934 001
Gene Knockdown Reagent		
X-tremeGENE siRNA Transfection Reagent	1 ml (400 transfections in a 24-well plate)	04 476 093 001
Mycoplasma Detection Reagents		
Mycoplasma PCR ELISA	1 kit (96 reactions)	11 663 925 910
Plasmid Isolation Products		
Genopure Plasmid Midi Kit	1 kit (for up to 20 preparations)	03 143 414 001
Genopure Plasmid Maxi Kit	1 kit (for up to 10 preparations)	03 143 422 001
Protease Inhibitor Tablets and Lysis Reagents		
cOmplete	20 tablets in glass vials 3 x 20 tablets in glass vials 20 tablets in *EASYpacks*	11 697 498 001 11 836 145 001 04 693 116 001
cOmplete, EDTA-free	20 tablets in a glass vial 3 x 20 tablets in glass vials 20 tablets in *EASYpacks*	11 873 580 001 05 056 489 001 04 693 132 001
cOmplete Lysis-M (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 complete Protease Inhibitor Cocktail Tablets)	04 719 956 001
cOmplete Lysis-M,EDTA-free (for mammalian cell lysis)	1 kit (200 ml lysis reagent and 20 complete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 964 001

Product	Pack Size	Cat. No.
Reporter Gene Assays		
CAT ELISA	1 kit (192 tests)	11 363 727 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit (500 assays, microplate format, 250 assays, tube format)	11 758 241 001
β-Gal ELISA	1 kit (192 tests)	11 539 426 001
hGH ELISA	1 kit (192 tests)	11 585 878 001
Luciferase Reporter Gene Assay, high sensitivity	200 assays 1,000 assays	11 669 893 001 11 814 036 001
SEAP Reporter Gene Assay, chemiluminescent	1 kit (500 assays, microplate format, or 250 assays, tube format)	11 779 842 001
Selection Antibiotics		
G-418 Solution	20 ml 100 ml	04 727 878 001 04 727 894 001
Hygromycin B	1 g (20 ml)	10 843 555 001
Transfection Reagents		
X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
Western Blotting Reagents		
Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1 kit (1,000 cm^2 membrane)	12 015 218 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml (1,000 cm^2 membrane)	12 015 196 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3.00 m)	03 010 040 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm^2) 6 × 100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
Cellular Imaging Kits		
Phospho Histone H3 Imaging Kit		06 569 161 001
DNA Fragmentation Imaging Kit		06 432 344 001
Cell Viability Imaging Kit		06 432 379 001
Cell Counters and Analyzers		
Cedex XS Analyzer with Control Unit		05 926 432 001
Cedex Smart Slide package	15 x 8 measurements	05 650 801 001
CASY Model TT 45, 60, 150 µm		05 651 735 001

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