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

X-tremeGENE 360 Transfection Reagent

Version: 03
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For transient and stable transfection of eukaryotic cells.

Cat. No. 08 724 105 001	0.4 ml
Cat. No. 08 724 121 001	1.0 ml
Cat. No. 08 724 156 001	5 x 1.0 ml

Store the reagent at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	white	X-tremeGENE 360 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. ▪ Does not contain any ingredients of human or animal origin.	08 724 105 001	1 vial, 0.4 ml
				08 724 121 001	1 vial, 1 ml
				08 724 156 001	5 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the reagent is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	white	X-tremeGENE 360 Transfection Reagent	Store at −15 to −25°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 −15 to −25°C.*** ⚠ ***Shipping temperature of this product is different from the storage temperature. This will not affect product performance or product stability.***

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

For siRNA preparation

- siRNA used for transfection should be highly pure, sterile, and the correct sequence. Depending on the type of experiment, the optimal final siRNA concentration for transfection is typically within the range of 10 to 50 nM. As a starting point, use 25 nM siRNA (final concentration in well).

Antibiotics

- Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in culture medium containing low levels of antibiotics (0.1 to 1x final concentration of penicillin/streptomycin mixture).

1.4. Application

X-tremeGENE 360 Transfection Reagent is a high performance transfection reagent, free of animal-derived components.

- Designed to transfect a broad range of eukaryotic cells,many cell lines not transfected well by other reagents, and hard-to-transfect cell lines, such as K-562 and HepG2.
- Can be successfully used in a variety of applications, such as gene expression analysisusing transiently transfected cells, generation of stable cell lines, expression of siRNA for gene knockdown studies, and CRISPR gene-editing tool.
- 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

Control Reactions

Controls for siRNA protocol

Transfect a non-targeting or nonsense siRNA control sequence to verify that the gene expression knockdown or phenotype is attributed to the gene-specific siRNA. Additionally, independent transfection of multiple siRNA sequences targeting a given gene minimizes the possibility that the observed phenotype is due to off-target effects.

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 360 Transfection Reagent 1 second prior to removing the required amount.
- Do not aliquot X-tremeGENE 360 Transfection Reagent; store in the original glass vials.
- Minimize the contact of undiluted X-tremeGENE 360 Transfection Reagent with plastic surfaces.
- The minimum amount of X-tremeGENE 360 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 360 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 360 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 360 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.

DNA transfection protocol

1. Allow X-tremeGENE 360 Transfection Reagent, DNA, and diluent to equilibrate to +15 to +25°C.
   – Briefly vortex the X-tremeGENE 360 Transfection Reagent vial.

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2. Dilute DNA with appropriate diluent, such as serum-free medium to a final concentration of 1 µg plasmid DNA/100 µl medium (0.01 µg/µl); mix gently.

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3. 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.***
   ℹ *Use sterile tubes or tissue culture-treated round-bottom, 96-well plates to produce the complex.*

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4. Pipette the X-tremeGENE 360 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 360 Transfection Reagent to come into contact with plastic surfaces. Do not use siliconized pipette tips or tubes.***

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5. 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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6. 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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7. 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.

CRISPR ribonucleoprotein transfection protocol

1. Allow X-tremeGENE 360 Transfection Reagent, Cas9 protein, RNA, and diluent to equilibrate to +15 to +25°C.
   – Briefly vortex the X-tremeGENE 360 Transfection Reagent vial.

2. Dilute a 50 µM guide RNA stock solution with appropriate diluent, such as serum-free medium to a final concentration of 12 nM per well using a sterile tube; mix gently by pipetting.
   If using 2-part crRNA + tracrRNA, combine at a 1:1 molar ratio and incubate for 10 minutes at +15 to +25°C to anneal. Then add to tube containing the appropriate diluent.

3. Add a 30 µM Cas9 protein stock solution to the guide RNA at a final concentration of 6 nm per well; mix gently by pipetting.
   A 2:1 ratio of guide RNA to Cas9 protein is a recommended starting point. Further optimization may be required.

4. Incubate the mixture for 10 minutes at +15 to +25°C to allow RNPs to form.

5. Add X-tremeGENE 360 Transfection Reagent to the RNP mixture; mix gently by pipetting.
   The amount of X-tremeGENE 360 Transfection Reagent needed may vary by cell type. The 2:1 ratio from the table in the section, **Guidelines for preparing Reagent:DNA complex for various culture vessel sizes** *is a good starting point.*

6. Incubate the transfection reagent:RNPcomplex mixture for 15 minutes at +15 to +25°C.

7. 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 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:RNA complex has been added to the cells, there is no need to replace with fresh medium as may be required with other transfection reagents.

8. Following transfection, incubate cells for 24 to 72 hours.
   – The duration of incubation will depend on many factors, including the transfected material, the cell type being transfected, the cell medium, cell density, and the target gene.
   – After the incubation period, measure the efficiency using an assay appropriate for your system.

siRNA transfection protocol

1. Allow X-tremeGENE 360 Transfection Reagent, siRNA, and diluent to equilibrate to +15 to +25°C.
   – Briefly vortex the X-tremeGENE 360 Transfection Reagent vial.

2. Dilute a 10 µM siRNA stock solution with Opti-MEM I Reduced Serum Medium to a final concentration of 25 nM per well using a sterile tube; mix gently by pipetting.
   – Dilute the siRNA using the manufacturer's recommended diluent.
   Alternatively, use 100 mM NaCl in 50 mM Tris, pH 7.5 (using RNase-free water). Do not use water alone to dilute siRNA, as this may result in denaturation of the siRNA at low concentrations.

3. Pipette the X-tremeGENE 360 Transfection Reagent directly into the medium containing the diluted siRNA without coming into contact with the walls of the plastic tubes; mix gently.
   The amount of X-tremeGENE 360 Transfection Reagent needed may vary by cell type. For a 6-well plate, use 7.5 µl as starting point. For further optimization, use three different volumes: 5, 7.5, and 10 µl per well. See also section, **Guidelines for preparing Reagent:siRNA complex for various culture vessel sizes** *to determine component amounts corresponding to the surface area of the cell culture vessel used.*

4. Incubate the transfection reagent:siRNA complex for 15 to 30 minutes at +15 to +25°C.

5 Add the transfection complex to the cells in a dropwise manner.
– 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:siRNA complex has been added to the cells, there is no need to replace with fresh medium as may be required with other transfection reagents. If required, perform a medium change at least 4 hours post-transfection.

6 Following transfection, incubate cells for 24 to 72 hours.
– The duration of incubation will depend on many factors, including the transfected material, the cell type being transfected, the cell medium, cell density, and the target gene.
– After the incubation period, measure the efficiency using an assay appropriate for your system.
– When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary, particularly if the target protein has a long cellular half-life.

Guidelines for preparing 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 using 3:1 or 2:1 ratio [µg]	Final amount of X-tremeGENE 360 Transfection Reagent using 3:1 ratio [µl]	Final amount of X-tremeGENE 360 Transfection Reagent using 2:1 ratio [µl]
96-well plate (1 well)	0.3	0.1	10	0.1	0.3	0.2
48-well plate (1 well)	1.0	0.3	30	0.3	0.9	0.6
24-well plate (1 well)	1.9	0.5	50	0.5	1.5	1
12-well plate (1 well)	3.8	1.0	100	1.0	3.0	2.0
35-mm dish	8.0	2.0	200	2.0	6.0	4.0
6-well plate (1 well)	9.4	2.0	200	2.0	6.0	4.0
60-mm dish	21	5.0	500	5.0	15.0	10
10-cm dish	55	10	1,000	10	30	20
T-25 flask	25	6.0	600	6.0	18.0	12
T-75 flask	75	20	2,000	20	60	40

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

Culture vessel	Surface area [cm²]	Growth medium [µl]	Serum-free medium [µl]	siRNA (10 µM stock) 25 nM final [µl]	Amount of X-tremeGENE 360 Transfection Reagent [µl]
96-well plate (1 well)	0.35	92	9	0.25	0.3
48-well plate (1 well)	1.0	263	26	0.7	0.78
24-well plate (1 well)	1.9	500	50	1.4	1.5
12-well plate (1 well)	3.8	1,000	100	2.8	3
6-well plate (1 well)	9.6	2,500	250	6.8	7.5
10-cm dish	59	15,500	1,500	42.5	45
T-75 flask	75	19,700	1,900	54	57

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 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 360 Transfection Reagent

To optimize, first transfect a monolayer of cells that is 70 to 90% confluent, using 1:1, 2:1, 3:1, and 4:1 ratios of microliter (µl) X-tremeGENE 360 Transfection Reagent to microgram (µg) DNA. A ratio of 3:1 of microliter (µl) X-tremeGENE 360 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 360 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, **Troubleshooting**.

3. Results

CHO-K1 cells were transfected with a GFP plasmid (Figure 1). CHO-K1 cells were observed under fluorescence and bright field microscopy at 63× magnification.

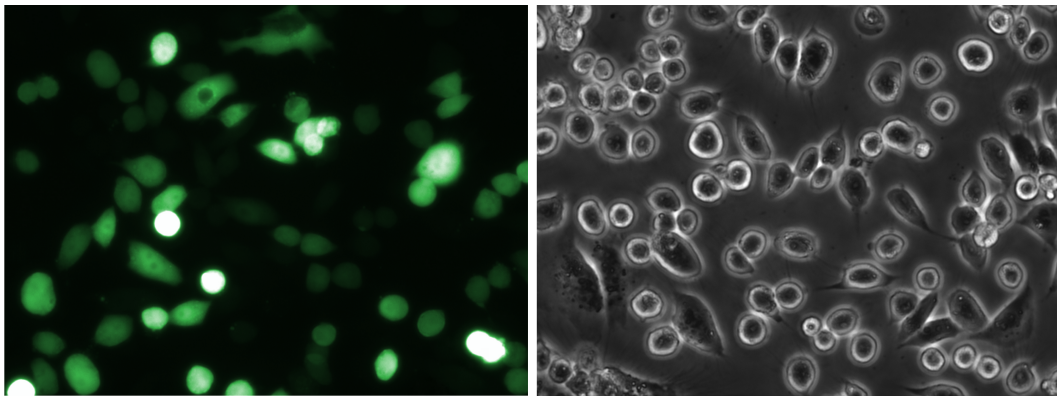

Fig. 1: X-tremeGENE 360 Transfection Reagent (3:1 ratio).
Different cell types were transfected with siRNA. The normalized level of expressions after transfection are shown in Figure 2.

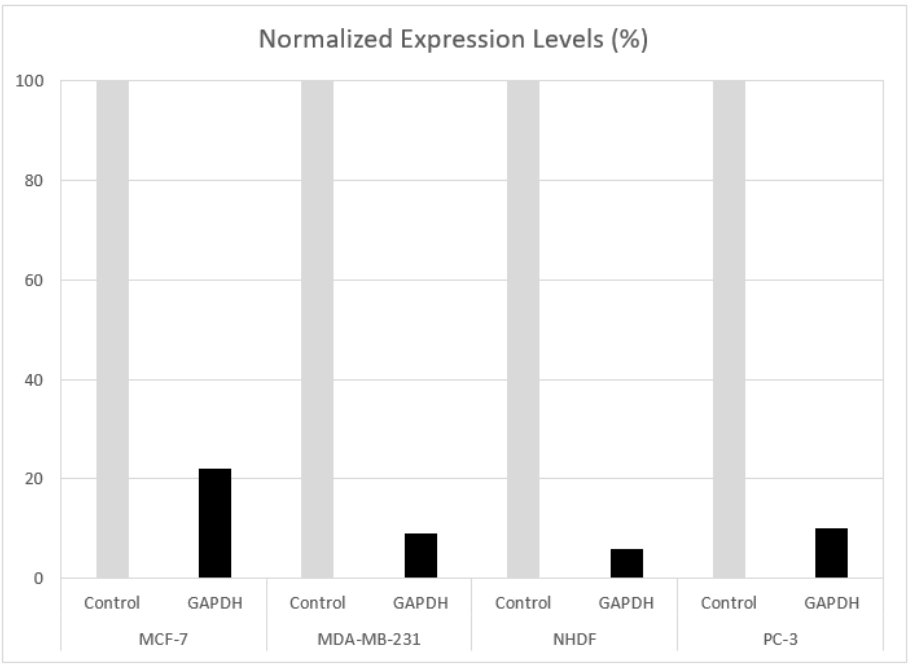

Fig. 2: Level of gene expression after GAPDH siRNA transfection.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low transfection efficiency.	Suboptimal X-tremeGENE 360 Transfection Reagent:DNA ratio.	Titrate optimal X-tremeGENE 360 Transfection Reagent:DNA ratio, see section, **General Considerations, Required amount of X-tremeGENE 360 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 360 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 360 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.
	Low volume of X-tremeGENE 360 Transfection Reagent:DNA complex.	The minimum amount of X-tremeGENE 360 Transfection Reagent to DNA complex is 100 µl. *Complex formation at lower volumes may significantly decrease the transfection efficiency, see section,* **General Considerations, Precautions**.
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 360 Transfection Reagent in cells cultured in serum-free medium is possible, however, toxicity may be higher when serum is absent.
	X-tremeGENE 360 Transfection Reagent:DNA complexes and cells not mixed well.	Add X-tremeGENE 360 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

Each lot of X-tremeGENE 360 Transfection Reagent is tested using established quality control procedures.
For lot-specific certificates of analysis, see section **Contact and Support**.

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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