3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

*Trans*IT-CRISPR® Transfection Reagent

Catalog Number **T1706**

Product Description

*Trans*IT-CRISPR is a non-liposomal polymeric transfection reagent for efficient delivery of CRISPR/Cas components. *Trans*IT-CRISPR allows for simple, fast and efficient delivery of CRISPR DNA, guide RNA and RNP (Cas9-gRNA ribonucleoprotein) complexes to various cell types, including primary cells. Also, *Trans*IT-CRISPR effectively delivers siRNA duplexes into various cell lines.

*Simple protocols for easy optimization in delivering CRISPR/Cas components to cells in DNA, guide RNA and RNP formats.

*Efficient delivery to various cell lines, including common cell types and difficult to transfect cell types.

Storage/Stability

*Trans*IT-CRISPR Transfection Reagent is shipped on wet ice. Upon receipt, please store at –20°C. With proper handling and storage, this product remains stable for one year from the date of purchase.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

SIGMA-ALDRICH CRISPR PLASMID DNA TRANSFECTION

Tips for Plasmid DNA Transfection

Optimize reaction conditions for different cell types to ensure successful transfections. The suggestions provided in this protocol yield high efficiency plasmid DNA transfection using the *Trans*IT-CRISPR Transfection Reagent. Table 1 lists recommended starting conditions depending on culture vessel size.

• **Cell density at transfection**:
The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Split cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.

• **DNA purity**:
Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have A260/280 absorbance ratio of 1.8–2.0 are desirable. DNA prepared using mini-prep kits is not recommended as it might contain high levels of endotoxin.

• **Ratio of *Trans*IT-CRISPR to DNA**: Determine the best *Trans*IT-CRISPR: DNA ratio for each cell type. Start with 3 µl of *Trans*IT-CRISPR per 1 µg of DNA. Vary the amount of *Trans*IT-CRISPR from 2–6 µl per 1 µg DNA to find the optimal ratio. Table 1 provides recommended starting conditions.

• **Complex formation conditions**: Prepare *Trans*IT-CRISPR: DNA complexes in serum-free growth medium. Recommendation: Opti-MEM™ I Reduced-Serum Medium, without antibiotics.

• **Cell culture conditions**:
Culture cells in the appropriate medium, with or without serum. There is no need to perform a medium change to remove the transfection complexes.

• **Presence of antibiotics**:
Transfection complexes can be added directly to cells grown in complete culture medium containing serum and low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture). Do not include antibiotics during the complex formation step.

• **Post-transfection incubation time**: Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment, nature of the plasmid used, and cell doubling time.

Sigma-Aldrich CRISPR Plasmid DNA Transfection Protocol per Well of a 6-Well Plate in Human Osteosarcoma (U2OS) Cells and a Human Immortalized Liver Cell Line (HepaRG)

The following procedure describes how to perform plasmid DNA transfections using the *Trans*IT-CRISPR transfection reagent in 6-well plates. The surface areas of other culture vessels are different and transfections must be scaled accordingly (see Table 1 as an example).

Plate Cells

- Approximately 18-24 hours before transfection, plate cells in 2.5 ml complete growth medium per well in a 6-well plate. For most cell types, cultures should be ≥ 80% confluent at the time of transfection.
    - *For adherent U2OS and HepaRG cells*: Plate at a density of 2.5 x 10^5 cells/ml.
    - *For other adherent cells*: Plate cells at a density of 0.8–3.0 × 10^5 cells/ml.
- Incubate cell cultures overnight.

Prepare *Trans*IT-CRISPR: DNA CRISPR complexes (Immediately before transfection)

- Warm *Trans*IT-CRISPR to room temperature and vortex gently before using.
- Place 250 µl of Opti-MEM® I Reduced – Serum Medium in a sterile tube.
- Add between 2 – 4 µg of Sigma-Aldrich CRISPR plasmid DNA.

Table 1. Recommended starting conditions for DNA transfections with *Trans*IT-CRISPR Transfection Reagent

DNA							
Culture vessel	96-well	48-well	24-well	12-well	6-well	10-cm dish	T75 flask
Surface area	0.35 cm^2	1 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2	75 cm^2
Complete growth medium	92 µl	263 µl	0.5 ml	1 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
DNA (1 µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl

For the all-in-one Sigma-Aldrich CRISPR FP vectors, add 4 µg of plasmid. For the dual vector Sigma-Aldrich CRISPR plasmids, add 2 µg of U6-gRNA only plasmid and 2 µg of CMV-Cas9-FP only plasmid.

- Pipet gently to mix completely.
- Add 3-6 µl *Trans*IT-CRISPR (1.5 µl of *Trans*IT-CRISPR per 1 µg of CRISPR plasmid DNA) to the diluted DNA mixture.
- Pipet gently to mix completely.
- Incubate at room temperature for 15-30 minutes to allow sufficient time for complexes to form.

Distribute the complexes to cells in complete growth medium

- Add the *Trans*IT-CRISPR: DNA complexes drop-wise to different areas of the wells.
- Gently rock the culture vessel back and forth and from side-to–side to evenly distribute the *Trans*IT-CRISPR: DNA complexes.
- Incubate for 48 hours. It is not necessary to replace the complete growth medium with fresh medium.
- Harvest cells and assay as required.

Delivery of purified recombinant Cas9 protein and guide RNA Ribonucleoprotein Complexes (RNPs

Recent papers have shown the efficacy of delivering the CRISPR components by combining purified Cas9 protein-guide RNA ribonucleoprotein (RNP) complexes. *Trans*IT-CRISPR transfection reagent has been tested in U2OS and HepaRG cells for efficient delivery of Cas9-gRNA RNP complexes to induce efficient site-specific mutations at targeted genomic regions. Protocols for both IVT gRNA and synthetic crRNA & tracrRNA are listed below.

Prepare *Trans*IT-CRISPR: *In Vitro* Transcribed gRNA/Cas9 Protein CRISPR complexes (Immediately before transfection)

Use between a 1.2 and 5 molar excess of *in vitro* transcribed RNA to Cas9 protein.

- Pipet between 1.2 and 5 µg of *in vitro* transcribed RNA to a sterile tube on ice.
- Add between 5 and 10 µg of Cas9 protein to the *in vitro* transcribed RNA, mix gently and incubate on ice for 30 minutes.
- Warm *Trans*IT-CRISPR to room temperature and vortex gently before using.
- Place 250 µl of Opti-MEM™ I Reduced –Serum Medium to the Cas9 Ribonucleoprotein (RNP) sterile tube.
- Add between 5 - 6.25 µl of *Trans*IT-CRISPR to the diluted Cas9 RNP.
- Pipet gently to mix completely.
- Incubate at room temperature for 15 – 30 minutes to allow sufficient time for complexes to form.

Prepare *Trans*IT-CRISPR: SygRNA™ Synthetic crRNA & tracrRNA /Cas9 Protein CRISPR complexes (Immediately before transfection)

Use a between a 1 and 5 molar excess of SygRNA™ synthetic crRNA & tracrRNA to Cas9 protein.

- Pipet between 30 and 300 pmol each of synthetic crRNA and tracrRNA into a sterile tube on ice (typically between 1.5 and 15 µl of 20 µM synthetic RNA stock solutions).
- Add between 5 and 10 µg of Cas9 protein (30 to 60 pmol) to the synthetic crRNA and tracrRNA, mix gently and incubate on ice for 30 minutes.
- Warm *Trans*IT-CRISPR to room temperature and vortex gently before using.
- Place 250 µl of Opti-MEM I Reduced – Serum Medium to the Cas9 RNP sterile tube.
- Add between 5 - 6.25 µl of *Trans*IT-CRISPR to the diluted Cas9 RNP.
- Pipet gently to mix completely.
- Incubate at room temperature for 15 – 30 minutes to allow sufficient time for complexes to form.

Distribute the complexes to cells in complete growth medium

- Add the *Trans*IT-CRISPR: RNP complexes drop – wise to different areas of the wells.
- Gently rock the culture vessel back-and-forth, and from side-to–side to evenly distribute the *Trans*IT-CRISPR: RNP complexes.
- Incubate for 24-72 hours. It is not necessary to replace the complete growth medium with fresh medium.
- Harvest cells and assay as required.

The *Trans*IT-CRISPR transfection reagent can also be used to deliver siRNA duplexes for gene silencing experiments. Increase knockdown efficiency by using *Trans*IT-CRISPR transfection reagent.

MISSION® siRNA TRANSFECTION

Important Tips for Optimal siRNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency knockdown of target gene expression using the *Trans*IT-CRISPR Transfection Reagent. For siRNA, please refer to Table 2 for recommended starting conditions depending on culture vessel size.

• **Cell density**: The recommended cell density for most cell types is ≥ 80% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Plate the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.

• **Volume of *Trans*IT-CRISPR**: Each cell type responds differently to a given transfection reagent.
As a starting point, test 7.5 µl of *Trans*IT-CRISPR per well of a 6-well plate. For further optimization, test three amounts of *Trans*IT-CRISPR, e.g. 5 µl, 7.5 µl, and 10 µl per well of a 6-well plate.

• **siRNA dilution**: Dilute siRNA using the manufacturer's recommended buffer. Alternatively, use 100 mM NaCl in 50 mM Tris, pH 7.5, made with RNase-free water. Do not use water alone to dilute siRNA, as this may result in denaturation of the siRNA at low concentrations.

• **siRNA concentration**: 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 10–50 nM. As a starting point, we recommend 25 nM siRNA (final concentration in well).

• **Proper controls**: We recommend transfecting a non-targeting siRNA (Sigma-Aldrich MISSION® siRNA Universal Negative Controls SIC001 or 6-FAM-labeled SIC007) to verify that the gene expression knockdown or phenotype is attributed to the gene-specific siRNA. Additionally, independent transfection of three to four siRNA duplexes targeting a particular gene minimizes the possibility that the observed phenotype is due to off-target effects.

• **Complex formation conditions**: Prepare *Trans*IT-CRISPR: siRNA complexes in serum-free growth medium. Recommended: Opti-MEM™ I Reduced-Serum Medium.

• **Cell culture conditions**: Culture cells in the appropriate medium, with or without serum There is no need to perform a medium change to remove the transfection complexes. *Trans*IT-CRISPR yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change.

• **Presence of 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 complete culture medium containing low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).

• **Transfection incubation time**: The optimal incubation time can be determined empirically by testing a range from 24–72 hours post-transfection, depending on the stability of the target mRNA and its encoded protein. When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post-transfection is often sufficient. 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.

Table 2. Recommended starting conditions for siRNA transfections with *Trans*IT-CRISPR Transfection Reagent

siRNA							
Culture vessel	96-well	48-well	24-well	12-well	6-well	10-cm dish	T75 flask
Surface area	0.35 cm^2	1 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2	75 cm^2
Complete growth medium	92 µl	263 µl	0.5 ml	1 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
MISSION® siRNA (10 µM stock) 25 nM final	0.25 µl	0.7 µl	1.4 µl	2.8 µl	6.8 µl	42.5 µl	54 µl
TransIT-CRISPR	0.3 µl	0.78 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

Related Products

- CRISPR Custom Plasmid and RNA, Catalog Number CRISPR SigmaAldrich.com/crisprs
- CRISPR CMV-CAS9-2A-GFP Plasmid, Catalog Number CAS9GFPP
- CRISPR CMV-CAS9D10A-2A-GFP Plasmid, Catalog Number CAS9D10AGFPP
- CRISPR Universal Negative Control 1, Catalog Number CRISPR06
- Custom SygRNA™, Catalog Number VC40003
- SygRNA™ Cas9 Synthetic tracrRNA, Catalog Number TRACRRNA05N-5NMOL
- Cas9-NLS from Streptococcus pyogenes, Catalog Number CAS9PROT
- Enhanced specificity Cas9-NLS from Streptococcus pyogenes, Catalog Number ESPCAS9PRO
- Cas9 Recombinant protein from Streptococcus pyogenes, Catalog Number TGEN-CP
- Cas9 Nickase Recombinant protein from Streptococcus pyogenes, Catalog Number TGEN-CNP
- MISSION® siRNA duplexes, predesigned and custom SigmaAldrich.com/siRNA
- MISSION® siRNA Universal Negative Control #1, Catalog Number SIC001
- MISSION® siRNA Fluorescent Universal Negative Control #1, 6-FAM, Catalog Number SIC007
- MISSION TRC 3 – LentiORF Collection SigmaAldrich.com/lentiorf

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