*Trans*IT®-Jurkat Transfection Reagent

Mirus The Transfection Experts

Protocol for MIR 2120, 2124, 2125, 2126

INTRODUCTION

*Trans*IT®-Jurkat Transfection Reagent is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in Jurkat cells and cell types of associated lineage. Jurkat cells (human T-lymphocyte; acute T-cell leukemia cell line) have been inherently difficult to transfect, yet have remained a prevalent cell line in the immunological research field. *Trans*IT-Jurkat reagent also works well for additional cell lines of hematopoietic origin such as THP-1(human acute monocytic leukemia cell line) and RAW264.7 (mouse monocyte/macrophage cell line). *Trans*IT-Jurkat reagent provides many attributes of the trusted *Trans*IT series of transfection reagents: high efficiency, serum compatibility, simplicity of use, and reproducibility. Transfection performed using the *Trans*IT-Jurkat Reagent does not require medium changes and can be carried out in serum-containing medium. *Trans*IT-Jurkat is suitable for both transient and stable transfection.

SPECIFICATIONS

Storage	Store *Trans*IT-Jurkat Reagent at 4°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

Warm *Trans*IT-Jurkat to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

*Trans*IT-Jurkat Transfection Reagent is supplied in ***one*** of the following formats.

Product No.	Quantity
MIR 2124	1 × 0.4 ml
MIR 2120	1 × 1.0 ml
MIR 2125	5 × 1.0 ml
MIR 2126	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified plasmid DNA
- Serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required
- *Optional:* Selection antibiotic (e.g., G418 or Hygromycin B) for stable transfection

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each Jurkat cell subtype to ensure successful transfections. The suggestions below yield high efficiency transfection of most Jurkat cell subtypes using *Trans*IT-Jurkat Transfection Reagent. **Table 1** presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** Determine the optimal cell density for each Jurkat cell subtype to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density (generally 4–8 $\times 10^5$ cells/ml) 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 $A_{260/280}$ absorbance ratio of 1.8–2.0 are desirable. DNA prepared using miniprep kits is not recommended as it may contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove endotoxin from your DNA preparation.

Do not use DNA prepared using miniprep kits for transfection.

- **Ratio of *Trans*IT-Jurkat Reagent to DNA.** Determine the best *Trans*IT-Jurkat Reagent:DNA ratio for each Jurkat cell subtype. Start with 3 µl of *Trans*IT-Jurkat Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-Jurkat Reagent from 1–5 µl per 1 µg DNA to find the optimal ratio. Additionally, increased protein expression has been observed when the amount of reagent and plasmid DNA are increased two-fold (e.g. 6 µl of *Trans*IT-Jurkat Reagent per 2 µg of DNA). **Table 1** provides recommended starting conditions based on cell culture vessel size.

Do not use serum or antibiotics in the medium during transfection complex formation.

Transfection complexes can be added directly to cells cultured

- **Complex formation conditions.** Prepare *Trans*IT-Jurkat Reagent:DNA complexes in serum-free growth medium. Mirus recommends 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.
- **Presence of antibiotics:** Antibiotics may inhibit transfection complex formation and therefore should be excluded from the complex formation step. 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 use serum or antibiotics in the medium during transfection complex formation.

- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each Jurkat cell subtype. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment, the nature of the plasmid, and the half-life of the expressed protein.

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

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10-cm dish	T75 flask
Surface area	0.35 cm^2	1.0 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	1.0 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 (1µg/µl stock)	0.1 µl	0.25 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-Jurkat Reagent	0.3 µl	0.75 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

Surface areas are based on Greiner tissue culture plates and Falcon 10-cm dishes and T75 flasks. All volumes given are per well (or per dish) for a given culture vessel.

If small volumes of *Trans*IT-Jurkat reagent need to be pipetted, dilute the reagent in 100% ethanol before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-Jurkat Reagent.

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections in 6-well plates. The surface areas of other culture vessels are different and transfections must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-Jurkat Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel (please refer to **Table 1** on Page 2).

Transient plasmid DNA transfection protocol per well of a 6-well plate

Divide cultured cells 18–24 hours before transfection to ensure active cell division at the time of transfection.

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells at a density of 2–4 $\times 10^5$ cells/ml in 2.5 ml complete growth medium per well in a 6-well plate.
2. Incubate cell cultures overnight.

   Optional: Alternatively, plate cells at a density of 4–8 $\times$ 10^5 cells/ml in 2.5 ml complete growth medium per well in a 6-well plate, just prior to transfection.

B. Prepare *Trans*IT-Jurkat Reagent:DNA complex (Immediately before transfection)

1. Warm *Trans*IT-Jurkat Reagent to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 2.5µg (2.5 µl of a 1 µg/µl stock) plasmid DNA. Pipet gently to mix completely.
4. Add 7.5µl *Trans*IT-Jurkat Reagent to the diluted DNA mixture. Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

Jurkat cells might become loosely adherent to tissue culture plates upon transfection. To collect these loosely adherent cells, pipet the cell mixture gently up and down.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-Jurkat Reagent:DNA complexes (prepared in Step B) drop-wise to different areas of the wells.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-Jurkat Reagent:DNA complexes.
3. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay as required.

There is no need to change fresh culture medium after transfection. If required, perform a medium change at least 4 hours post-transfection.

For generating stable cell transfectants, passage the cells 24–48 hours post-transfection in complete growth medium containing the appropriate selection antibiotic such as G418 or Hygromycin B. Maintain selection for 1–2 weeks, allowing selection of cells that have undergone stable integration of DNA.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
*Trans*IT-Jurkat Reagent was not mixed properly	Warm *Trans*IT-Jurkat to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-Jurkat Reagent:DNA ratio	Determine the best *Trans*IT-Jurkat Reagent:DNA ratio for each Jurkat cell subtype. Titrate the *Trans*IT-Jurkat Reagent from 1–5 µl per 1 µg DNA. Additionally, increased protein expression has been observed when the amount of reagent and plasmid DNA are increased two-fold (e.g. 2-10 µl of *Trans*IT-Jurkat Reagent per 2 µg of DNA). Refer to “Before You Start” on Page 2. **Note**: Increased toxicity may be observed when using higher amounts of *Trans*IT-Jurkat and DNA. We suggest noting cell health and viability prior to increasing the amount of reagent and DNA above the recommended starting conditions.
Suboptimal DNA concentration	Confirm DNA concentration and purity. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8–2.0.
	The optimal DNA concentration generally ranges between 1–3 µg/well of a 6-well plate. Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of *Trans*IT-Jurkat Transfection Reagent accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection.
	We recommend using Mirus Bio’s MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells.
	Do not use DNA prepared using miniprep kits as it may contain high levels of endotoxin.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-Jurkat Reagent:DNA complexes in serum-free growth medium. We recommend Opti-MEM I Reduced-Serum Medium. Once transfection complexes are formed, they can be added directly to cells cultured in complete growth medium containing serum and 0.1–1X antibiotics.
	Polyanions such as dextran sulfate or heparin can inhibit transfection. Use culture medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hours post transfection.
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Transfection incubation time	Determine the optimal transfection incubation time for each Jurkat cell subtype and experiment. Test a range of incubation times (e.g.12–72 hours). The best incubation time is generally 24–48 hours.
Cells not actively dividing at the time of transfection	Divide the culture at least 18–24 hours before transfection to ensure that the cells are actively dividing and reach optimal cell density at time of transfection.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents according to Table 1 on page 2 including serum-free medium, *Trans*IT-Jurkat and plasmid DNA.
	Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold.

TROUBLESHOOTING GUIDE continued

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY continued	
Proper experimental controls not included	To verify efficient transfection, use *Trans*IT-Jurkat Reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.
	To assess delivery efficiency of plasmid DNA, use Mirus' *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid **or** Mirus' prelabeled *Label* IT Plasmid Delivery Controls (please refer to Related Products on Page 6).
Consider electroporation for high efficiency nucleic acid delivery	Mirus also offers Ingenio™ Electroporation Kits and Solution for higher efficiency nucleic acid delivery into hard to transfect cell types such as Jurkat, THP-1, RAW264.7 and K562, etc. (please refer to Related Products on Page 6).
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-Jurkat Reagent:DNA complexes drop-wise to different areas of the wells containing cells. Gently rock the dish back-and-forth and from side-to-side to distribute the complexes evenly. Do not swirl or rotate the dish, as this may cause uneven distribution.
Transfection complexes added to cells cultured in serum-free medium	Allow *Trans*IT-Jurkat Reagent:DNA complexes to form in serum-free medium, then add these complexes to cells cultured in complete growth medium. The presence of serum in the growth medium improves transfection efficiency and reduces cytotoxicity. No culture medium change is required after the addition of transfection complexes to cells.
Endotoxin-contaminated plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection.
	We recommend using Mirus Bio's MiraCLEAN Endotoxin Removal Kit (MIR 5900) for removal of any traces of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells.
	Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin.
Expressed target gene is toxic to cells	Compare toxicity levels against a cells alone control and cells transfected with an empty vector to assess the cytotoxic effects of the target protein being expressed.
	If lower levels of target gene expression are desired in your transfection experiments, consider reducing the amount of target plasmid. Maintain the optimal *Trans*IT-Jurkat:DNA ratio by using carrier DNA such as an empty cloning vector.
Cell density not optimal at time of transfection	Determine the best cell density for each Jurkat cell subtype to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most Jurkat cell subtypes, a cell density of $4–8 \times 10^5$ cells/ml is recommended at transfection, but use of higher or lower densities may increase cell viability depending on subtype.
Cell morphology has changed	Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check your cells for Mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate Mycoplasma.
	A high or low cell passage number can make cells more sensitive and refractory to transfection. Maintain a similar passage number between experiments to ensure reproducibility.

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RELATED PRODUCTS

- Ingenio Electroporation Solution and Kits
- *Label* IT Plasmid Delivery Controls
- *Label* IT Tracker Intracellular Nucleic Acid Localization Kits
- MiraCLEAN Endotoxin Removal Kits
- *Trans*IT Cell Line Specific Transfection Reagents and Kits
- *Trans*IT-2020 Transfection Reagent
- *Trans*IT-PRO™ Transfection Kit
- *Trans*IT-*In Vivo* Gene Delivery Kits
- *Trans*IT-LT1 Transfection Reagent
- *Trans*IT-QR and *Trans*IT-EE Delivery Solutions and Kits

For details on our products, visit www.mirusbio.com or www.TheTransfectionExperts.com.

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Use of Mirus Bio *Trans*IT® polyamine transfection reagents are covered by U.S. Patent No. 5,744,335, No. 6,180,784, No. 7,101,995, No. 7,601,367 and patents pending. Mirus Bio *Label* IT® nucleic acid labeling and modifying reagents are covered by U.S. Patent No. 6,262,252, No. 6,593,465, No. 7,049,142, No. 7,326,780 and No. 7,491,538. Cy™3 and Cy™5 products or portions thereof are manufactured under license from Carnegie Mellon University and are covered by U.S. Patent No. 5,268,486.

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