*Trans*IT®-Jurkat Transfection Reagent

Product # MIR 2120, MIR 2124, MIR 2125, MIR 2126

Product Name	Volume of *Trans*IT®-Jurkat Reagent	Product No.
*Trans*IT®-Jurkat Transfection Reagent	0.4 ml	MIR 2124
	1 ml	MIR 2120
	5 ml (5 × 1 ml)	MIR 2125
	10 ml (10 × 1 ml)	MIR 2126

1.0 INTENDED USE

The *Trans*IT®-Jurkat Transfection Reagent was specifically developed to obtain maximal transfection efficiency in Jurkat cells. MIR 2120 (1 ml) provides sufficient reagent to perform up to 500 transfections in 35 mm wells.

2.0 DESCRIPTION

2.1 General Information

The *Trans*IT®-Jurkat Transfection Reagent was developed by the gene transfer specialists of Mirus Bio Corporation. This novel reagent is specifically optimized to provide superior transfection efficiency in Jurkat cells. Jurkat cells (human T-lymphocyte; acute T-cell leukemia) have been inherently difficult to transfect, yet have remained a prevalent cell line in the immunological research field. The specificity of the *Trans*IT®-Jurkat Transfection Reagent makes this product a desirable alternative to broad spectrum transfection reagents. The reagent provides many attributes of the trusted *Trans*IT® Reagent line: high efficiency, simplicity of use, and reproducibility. Transfections with the *Trans*IT® Reagents do not require media changes and can be carried out in serum-containing media. In addition, *Trans*IT®-Jurkat Transfection Reagent is quality control tested on ATCC Jurkat cells. These significant features establish the *Trans*IT®-Jurkat Transfection Reagent as the product of choice for transfecting Jurkat cells.

2.2 Specifications

Concentration: *Trans*IT®-Jurkat Reagent: 10 mg/ml in 100% ethanol

Storage: Store the TransIT®-Jurkat Reagent at 4˚C. Prior to use, warm it to room temperature and gently vortex to dissolve any precipitate that may have formed.

Stability: 6 months from the date of purchase when stored properly

3.0 PROCEDURE

3.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each Jurkat subtype. The transfection protocol described in Section 3.2 should result in efficient transfection of most Jurkat cell subtypes; however, to ensure optimal results, consider the following variables:

A. Cell density at transfection - The recommended cell density for most Jurkat cell types at transfection is 4-8 x 10^5 cells/ml. Determine the optimal cell density for each Jurkat cell subtype in order to maximize transfection efficiency. Maintain this in future experiments for reproducibility.

B. DNA purity and concentration for transfection - DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is within the range of 1-3 µg per 35 mm well. As a starting point, use 2 µg per 35 mm well.

C. *Trans*IT®-Jurkat Reagent to DNA ratio - As a starting point, use 2-4 µl of the *Trans*IT®-Jurkat Reagent per 1 µg of DNA. The optimal TransIT®-Jurkat Reagent to DNA ratio can be determined by titrating the reagent starting at 1-5 µl per µg DNA. For future transfections, use the ratio that gives the best transfection efficiency with the lowest cellular toxicity, on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

D. Transfection Incubation Time – Determine the optimal incubation time empirically by testing a range of incubation times from 4-48 hours.

The protocol below is recommended for performing transfections with the *Trans*IT®-Jurkat Transfection Reagent in 35 mm wells. When performing transfections in different sized wells, the amounts of DNA, *Trans*IT®-Jurkat Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish. The following table recommends starting conditions:

Table 1. Recommended starting conditions for using the TransIT®-Jurkat Transfection Reagent:

Culture Vessel	6-well (35 mm) plate	12-well plate	24-well plate
Surface Area	9.4 cm^2	3.8 cm^2	1.9 cm^2
Serum-free Media	200 µl	100 µl	50 µl
*Trans*IT®-Jurkat Reagent	4-8 µl	2-4 µl	1-2 µl
DNA (1µg/µl stock)	2 µl	1µl	0.5 µl
Complete Growth Media	2000 µl	1000 µl	500 µl

*All volumes in Table 1 are per one well of indicated size.

3.2 Protocol for Transient Transfection (in 35 mm wells)

A. Cell Plating

1. The day of transfection, plate cells in complete growth medium at 4-8 x 10^5 cells/ml using 2 ml per 35 mm well. Cells can also be plated the day before transfection at half the desired density.
2. Incubate the cells until time of transfection.[b]

B. Complex Formation (perform this procedure immediately prior to transfection)

1. In a sterile plastic tube, add the *Trans*IT®-Jurkat Reagent (1-5 µl per µg DNA; see Table 1) dropwise into 200 µl of serum-free medium[c,e]. Mix thoroughly by pipetting.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1-3 µg per well; see Table 1) to the diluted *Trans*IT®-Jurkat Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-Jurkat Transfection Reagent yields improved transfection efficiencies when transfections are performed in complete growth medium (as compared to serum-free medium) and the media change is eliminated.

1. If necessary, spin down the cells prepared in step A, remove the medium from these cells, and replace it with 2 ml per 35 mm well (see Table 1) of fresh complete growth medium. Replate cells as described in Section 3.2A.
2. Add the *Trans*IT®-Jurkat Reagent/DNA complex mixture prepared in step B dropwise to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 24-48 hours. [b]

   NOTE: The above incubation is designed for transfections performed with no media change. To perform a media change after a 4-24 hour incubation with the complexes, collect cells by centrifugation, replace the original medium with fresh complete growth medium, mix, plate cells, and incubate for an additional 24-48 hours.[b,d]
4. Harvest cells and assay as needed.

   NOTE: When suspension cells are transfected with the *Trans*IT®-Jurkat Reagent, they may become loosely adherent to tissue culture plates. When harvesting, the cells will dislodge easily by pipetting the cell mixture up and down across the surface of the bottom of the well.

[a] Since the optimal cell density for efficient transfection can vary between Jurkat cell subtypes, maintain the same seeding protocol for subsequent experiments.

[b] Standard incubation conditions for Jurkat cells are 37˚C in 5% CO_2.

[c] The *Trans*IT®-Jurkat Reagent/DNA complex may form improperly if the complex formation medium contains serum, resulting in poor transfection efficiencies.

[d] The optimal incubation time can be determined empirically by testing a range of incubation times from 4-48 hours.

[e] For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 200-1,000 µl.

4.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-Jurkat Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-Jurkat Reagent to DNA ratio by titrating the reagent from 1-5 µl per µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 2-4 µl of *Trans*IT®-Jurkat Reagent per 1 µg of DNA.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (serum-containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to the cells in complete growth media and the media change is eliminated.
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**
  Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (LPS) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT®-Jurkat Reagent/DNA complex formation**
  Use serum-free medium when forming the complexes. Transfections should be performed in the presence of serum.
- **Cell density not optimal at time of transfection**
  The recommended cell density for most Jurkat cell types at the time of transfection is 4-8 x 10^5 cells/ml. However, it may be necessary to determine the optimal cell density for specialized experiments in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, transfection efficiency can be adversely affected. To ensure reproducibility, maintain a similar passage number between experiments.
- **Inhibitor present during transfection**
  The presence of polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use transfection medium that does not contain these polyanions.

High Cellular Toxicity

- **Excessive amounts of *Trans*IT®-Jurkat Reagent or DNA were used in transfection**
  Reduce the amount of appropriate reagent in the transfection. See Table 1 for recommended starting conditions.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (serum-containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to the cells in complete growth media and the media change is eliminated.
- ***Trans*IT®-Jurkat Reagent/DNA complex mixture was not mixed thoroughly following addition to the cells**
  Mix thoroughly to evenly distribute the complexes to all cells. Rocking the dish back and forth and from side to side is recommended. Do not swirl or rotate the dish, as this may result in uneven distribution.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, they can be more sensitive to transfection reagents. To ensure reproducibily, maintain a similar passage number between experiments.
- **Cell density was too low at time of transfection**
  Grow cells to a higher cell density and repeat the experiment.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801 or techsupport@mirusbio.com.
For a list of citations using Mirus Bio products, please visit the Technical Resources section of our website (www.mirusbio.com).

5.0 RELATED PRODUCTS

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

Transfection reagents:*

*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-293 Transfection Kit (Product # MIR 2700)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-mRNA Transfection Reagent (Product # MIR 2250)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo* Gene Delivery Kits:

*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)
*Trans*IT®-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
*Trans*IT®-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)
*Trans*IT®-QR Hydrodynamic Delivery Solution (Product # MIR 5240)
*Trans*IT®-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

RNA Interference Products:*

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
siXpress® PCR Vector Systems (Product # MIR 7300, 7301, 7302)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT-TKO® Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®-siQUEST™ Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

*These products are available in additional sizes.

Mirus Bio Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811; 6,593,465 and patents pending.
The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.
This product is sold to the Buyer with a limited license to use this product for research only. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio Corporation.
*Trans*IT, *Trans*IT-TKO, *Trans*IT-Neural, MiraCLEAN, HeLaMONSTER, siXpress, and *Label* IT are registered trademarks of Mirus Bio Corporation.
Label IT Tracker and siQUEST are trademarks of Mirus Bio Corporation.