*Trans*IT®-Prostate Transfection Kit

Product Name	Volume of *Trans*IT®-Prostate Reagent	Volume of Prostate Boost Reagent	Product No.
*Trans*IT®-Prostate Transfection Kit	0.4 ml	0.56 ml	MIR 2134
	1 ml	1.4 ml	MIR 2130
	5 ml (5 × 1 ml)	7 ml (5 × 1.4 ml)	MIR 2135
	10 ml (10 × 1 ml)	14 ml (10 × 1.4 ml)	MIR 2136

Each millimeter of *Trans*IT®-Prostate Transfection Kit (MIR 2130) provides sufficient amounts of both reagents to perform up to 500 transfections in 6-well plates.

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT®-Prostate Transfection Kit was developed by the nucleic acid delivery specialists of Mirus Bio Corporation. This novel kit is specifically optimized to provide superior transfection efficiency in prostate cells without sacrificing cellular health. Generally, prostate cell types have been moderately difficult to transfect, yet have remained a prevalent cell line in the cancer research field. The specificity of the *Trans*IT®-Prostate Transfection Kit makes this product a desirable alternative to broad spectrum transfection reagents. The kit provides all the attributes of the trusted *Trans*IT® Reagent line: high efficiency, low toxicity, 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, the *Trans*IT®-Prostate Transfection Kit is quality control tested on ATCC LNCaP cells. These significant features establish the *Trans*IT®-Prostate Transfection Kit as the product of choice for transfecting prostate cells.

1.2 Cell Lines Successfully Tested by Mirus Corporation

DU 145, LNCaP, and PC-3 cells

1.3 Specifications

Concentration: *Trans*IT®-Prostate Reagent: 1.33 mg/ml in 80% ethanol
Prostate Boost Reagent: 1 mg/ml in 80% ethanol

Storage: Store both reagents at -20˚C. Prior to use, warm both reagents to room temperature and gently vortex to dissolve any precipitate that may have formed.

Stability: 6 months from the date of purchase when stored at -20˚C

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each prostate cell type. The transfection protocol described in Section 2.2 should result in efficient transfection of most prostate cell subtypes; however, to ensure optimal results, consider the following variables:

A. Media conditions - The *Trans*IT®-Prostate Kit yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection media change.

B. Cell density (% confluence) at transfection - The recommended cell density for most cell types at transfection is 50-70% confluence. Determine the optimal cell density for each prostate cell subtype in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.

C. DNA purity and concentration for transfection - DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin (lipopolysaccharides). Remove any traces of endotoxin using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product# MIR 5900). The optimal DNA concentration for transfection is within the range of 1 to 3 µg per well of a 6-well plate. As a starting point, use 2 µg per well of a 6-well plate.

D. *Trans*IT®-Prostate Reagent to DNA ratio - As a starting point, use 2 µl of *Trans*IT®-Prostate Reagent per 1 µg of DNA. The optimal *Trans*IT®-Prostate Reagent to DNA ratio can be determined by titrating the reagent from 2 to 5 µl per 1 µg of 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.

E. Prostate Boost Reagent to DNA ratio – Titrate the Prostate Boost Reagent from 0 to 5 µl per 1 µg of DNA depending on the cell type. For example, we find increased transfection efficiencies on LNCaP cells when 2 to 5 µl of Prostate Boost Reagent is added per 1 µg of DNA, whereas 0 to 2 µl of Prostate Boost Reagent per 1 µg of DNA is optimal for DU 145 cells. As a starting point, use 0, 2, and 4 µl of Prostate Boost Reagent per 1 µg of 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.

F. Transfection Incubation Time - The optimal incubation time can be determined empirically by testing a range of incubation times from 24-48 hours.

The protocol below is recommended for performing transfections in 6-well plates. When performing transfections in different sized wells, the amount of DNA, *Trans*IT®-Prostate Reagent, Prostate Boost Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish. To accommodate small pipetting volumes, the Prostate Boost Reagent can be diluted in 80% ethanol, immediately before use. Dilute only if necessary, and dilute only the necessary amount of Boost Reagent. Discard any unused diluted Prostate Boost Reagent.

Table 1. Recommended starting conditions for using the *Trans*IT®-Prostate Transfection Kit:

Culture Vessel	24-well plate	12-well plate	6-well plate
Surface Area	1.9 cm^2	3.8 cm^2	9.4 cm^2
Serum-free Media	50 µl	100 µl	200 µl
*Trans*IT®-Prostate Reagent	1-2.5 µl	2-5 µl	4-10 µl
DNA (1µg/µl stock)	0.5 µl	1 µl	2 µl
Prostate Boost Reagent (1µg/µl stock)	0-2.5 µl*	0-5 µl*	0-10 µl*
Complete Growth Media	500 µl	1000 µl	2000 µl

NOTE: All volumes in Table 1 are per one well of indicated size.
*Different Prostate Boost Reagent amounts are optimal for different prostate cell types. It may be necessary to titrate the Prostate Boost Reagent from 0 to 5 µl per 1 µg of DNA.

2.2 Protocol for Transient Transfection (in 6-well plates)

A. Cell Plating

NOTE: LNCaP cells are loosely adherent, and can become easily dislodged from tissue culture plates. Use poly lysine coated tissue cultures plates to aid in cell adherence.

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-2 x 10^5 cells in complete growth medium per well of a 6-well plate) to obtain ~50-70% confluency the following day. [a]
2. Incubate the cells overnight.[b]

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

1. In a sterile, plastic tube, add the *Trans*IT®-Prostate Reagent (2 to 5 µl per 1 µg of DNA) directly into 200 µl of serum-free medium.[c,e] Mix thoroughly by pipetting.
2. Incubate at room temperature for 5 to 20 minutes.
3. Add DNA (1 to 3 µg) to the diluted *Trans*IT®-Prostate Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 5 to 20 minutes.
5. Add Prostate Boost Reagent (0 to 5 µl per 1 µg of DNA) to the diluted *Trans*IT®-Prostate Reagent/DNA mixture and mix by gentle pipetting.
6. Incubate at room temperature for 5-20 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

1. If necessary, remove the medium from the cells prepared in step A and replace it with 2 ml per well of fresh complete growth medium.
2. Add the *Trans*IT®-Prostate Reagent/DNA/Prostate Boost Reagent 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. Do not swirl.
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, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.[b,d]
4. Harvest cells and assay for gene expression.

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[a] Since the optimal cell density (% confluence) for efficient transfection can vary between prostate cell subtypes, maintain the same seeding protocol for subsequent experiments.

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

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

[d] The optimal incubation time can be determined empirically by testing a range of incubation times from 24-48 hrs.

[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.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-Prostate Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-Prostate Reagent to DNA ratio by titrating the reagent from 2 to 5 µl per 1 µg of DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 3 µl of *Trans*IT®-Prostate Reagent per 1 µg of DNA in 6-well plates. Use 2 µg of DNA per well of a 6-well plate.
- **Suboptimal amounts of Prostate Boost Reagent**
  Determine the optimal Prostate Boost Reagent to DNA ratio by titrating the reagent from 0 to 5 µl per 1 µg of DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 0, 2, and 4 µl of Prostate Boost Reagent per 1 µg of DNA.
- **Cell morphology has changed**
  If the cell passage number is too high or too low the transfection efficiency may be adversely affected. Mirus recommends maintaining a similar passage number between experiments to maintain reproducibility.
- **Complexes were added to cells in serum-free media**
  Form complexes in serum-free media then 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.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most prostate cell types at the time of transfection is 50-70% confluence. 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.
- **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 using Mirus' MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT®-Prostate Reagent/DNA/Prostate Boost Reagent complex formation**
  Use serum-free medium when forming the complexes. Transfections should be performed in the presence of serum.
- **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

- **Complexes were added to 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.
- **Cell density (% confluence) was not optimal at time of transfection**
  The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments to ensure reproducibility.
- ***Trans*IT®-Prostate Reagent/DNA/Prostate Boost Reagent complex mixture was not mixed thoroughly with the cells in the well plate**
  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.
- **Excessive amounts of *Trans*IT®-Prostate Reagent, DNA, or Prostate Boost Reagent, were used in transfection**
  Reduce the amount of appropriate reagent in the transfection. See Table 1.
- **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. Maintain a similar passage number between experiments to ensure reproducibility.

For specific questions or concerns, please contact Mirus Bio' Technical Support at 888.530.0801 or techsupport@mirusbio.com.

For citations in which Mirus Bio' products have been used, please visit the Technical Resources Section of our website (www.mirusbio.com).

4.0 RELATED PRODUCTS

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

For endotoxin removal from DNA:*

MiraCLEAN® Endotoxin Removal Kit (Product #5900)

For DNA tracking applications:

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010, 7011, 7012, 7013, 7014, 7015)

Additional transfection reagents:*

*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)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-293 Transfection Reagent (Product # MIR 2700)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)

In Vivo* Gene Delivery Kits:

*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)

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)
*Trans*IT-TKO® HTS-96 Plates (Product # MIR 2530, 2540, 2550, 2560, 2570)
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.