*Trans*IT®-Oligo Transfection Reagent

Product # MIR 2160, MIR 2164, MIR 2165, MIR 2166

Product Name	Volume of *Trans*IT®-Oligo Reagent	Product No.
*Trans*IT®-Oligo Transfection Reagent	0.4 ml	MIR 2164
	1 ml	MIR 2160
	5 ml (5 × 1 ml)	MIR 2165
	10 ml (10 × 1 ml)	MIR 2166

1.0 INTENDED USE

*Trans*IT®-Oligo Transfection Reagent was developed by the gene transfer specialists of Mirus Bio Corporation. *Trans*IT®-Oligo Reagent efficiently and conveniently transfects oligos into a variety of cell types. Each milliliter of *Trans*IT®-Oligo Reagent (# MIR 2160) is sufficient quantity to perform up to 500 transfections in 24-well plates, depending on the specific cell type.

2.0 DESCRIPTION

2.1 General Information

Researchers have traditionally used transfection reagents developed for plasmid DNA delivery to transfect oligos *in vitro* with limited success. In response, Mirus Bio has developed *Trans*IT®-Oligo Transfection Reagent, specifically designed for efficient transfection of a wide range of oligonucleotides and oligoribonucleotides. *Trans*IT®-Oligo provides high transfection efficiency with the same ease-of-use and reproducibility found in the rest of the *Trans*IT® line of products. In addition, transfections using the *Trans*IT®-Oligo Reagent do not require media changes and can be carried out in serum-containing media.

2.2 Cell Lines and Oligonucleotides Successfully Tested by Mirus Corporation

Cell lines tested: CHO, COS-7, HeLa, HeLa-luc 705, MCF-7, NIH 3T3

Oligonucleotides tested: DNA, sDNA, 2'OMe RNA, Morpholino with DNA complement

2.3 Specifications

Concentration: 2.5 mg/ml in 100% ethanol

Storage: Tightly capped at 4˚C; DO NOT FREEZE

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 individual cell type. The transfection protocol described in Section 3.2 should result in efficient transfection of most cell types; however, to ensure optimal results the following variables should be considered:

A. Cell density (confluence) at transfection—The recommended cell density for most cell types at transfection is 50-70% confluence. The optimal cell density should be determined for each cell type in order to maximize transfection efficiency. This density should be maintained in future experiments for reproducibility.

B. Oligonucleotide concentration for transfection—Oligos used for transfection should be highly purified, sterile, and the correct sequence. The optimal final 2'OMe RNA concentration for transfection should be within the range of 0.5-5 µM in a 24-well plate (1.9 cm^2 dish). As a starting point, use 2 µM per 1.9 cm^2 dish. Refer to Table 1 for recommended starting conditions. The optimal final sDNA concentration for transfection should be within the range of 50-200 nM in a 24-well plate (1.9 cm^2 dish). As a starting point, use 100 nM per 1.9 cm^2 dish. Refer to Table 2 for recommended starting conditions.

C. *Trans*IT®-Oligo Reagent —As a starting point, use 3 µl of *Trans*IT®-Oligo Reagent per well of a 24-well plate. The optimal *Trans*IT®-Oligo Reagent concentration can be determined by titrating the reagent from 2 to 5 µl per well. The volume of reagent that gives the highest efficiency with the lowest cellular toxicity should be used for future transfections. Refer to Tables 1 and 2 for recommended starting conditions.

D. Transfection Incubation Time— The optimal incubation time can be determined empirically by testing a range of incubation times from 8-48 hours. Incubation times will vary according to the experiment being performed.

The protocol below is recommended for performing transfections with the *Trans*IT®-Oligo Transfection Reagent in 24-well plates. When performing transfections in different sized dishes, the amounts of oligonucleotide, *Trans*IT®-Oligo Reagent, and culture medium should be scaled up or down in proportion to the surface area of the well.

Table 1. Recommended starting conditions for using TransIT®-Oligo Reagent with 2'OMe RNA.

Culture Vessel:	96-well plate	24-well plate	12-well plate	6-well plate
Surface Area	0.32 cm^2	1.9 cm^2	3.8 cm^2	9.4 cm^2
TransIT®-Oligo Reagent	0.5-2 µl	2-5 µl	4-7 µl	8-12 µl
Complete Growth Media	100 µl	250 µl	500 µl	1000 µl
Serum-free Media	25 µl	50 µl	100 µl	200 µl
100 µM stock 2'OMeRNA (0.5-5 µM final concentration in well)	0.63-6.3 µl	1.5-15 µl	3.0-30 µl	6.0-60 µl

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

Table 2. Recommended starting conditions for using TransIT®-Oligo Reagent with sDNA

Culture Vessel:	96-well plate	24-well plate	12-well plate	6-well plate
Surface Area	0.32 cm^2	1.9 cm^2	3.8 cm^2	9.4 cm^2
TransIT®-Oligo Reagent	0.5-2 µl	2-5 µl	4-7 µl	8-12 µl
Complete Growth Media	100 µl	250 µl	500 µl	1000 µl
Serum-free Media	25 µl	50 µl	100 µl	200 µl
10 µM stock sDNA (50-200 nM final concentration in well)	0.63-2.50 µl	1.5-6.0 µl	3.0-12 µl	6.0-24 µl

*All volumes in Table 2 are per one well of indicated size

3.2 Oligonucleotide Transfection in 24-well plates

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~5 x 10^4 cells in complete growth medium per 1.9 cm^2 well) so that they will be ~50-70% confluent 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®-Oligo Transfection Reagent (1 to 5 µl- see Tables 1 and 2) dropwise into ≥50 µl of serum-free medium.[c,d] Mix thoroughly by vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add oligonucleotide (see Tables 1 and 2) to the diluted *Trans*IT®-Oligo Reagent. Mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.

C. Cell Preparation for Transfections in Complete Growth Medium

Note: For several cell lines tested, we have found that the *Trans*IT®-Oligo Reagent yields improved transfection efficiencies when the transfections are performed in complete growth medium (instead of serum-free medium) without a media change following transfection.

1. If necessary, adjust the volume in the well to 250 µl of complete growth media. (see Tables 1 and 2)
2. Add the *Trans*IT®-Oligo Reagent/oligonucleotide 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 8-48 hours[b].
   Note: The above incubation is designed for transfections performed with no media change. To perform a media change, remove the transfection complexes, replace the original medium with fresh complete growth medium and incubate for an additional 24-48 hours.[b,e]
4. Harvest for functional assay.

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

[b] Standard incubation conditions for mammalian cells are 37˚C in 5% CO_2. Other cell types, such as insect cells, require different temperatures and CO_2 concentrations. Use conditions appropriate for the cell type of interest.

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

[d] For transfecting concentrations of oligonucleotide beyond 20 µM or if a precipitate forms upon adding the reagent, use the highest recommended amount of *Trans*IT®-Oligo Reagent (see Tables 1 and 2) and increase the volume of serum-free medium two-fold.

[e] The optimal incubation time can be determined empirically by testing a range of incubation times from 8-48 hr.

4.0 Troubleshooting

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-Oligo Reagent**
  Determine the optimal *Trans*IT®-Oligo Reagent concentration by titrating the reagent from 1 µl to 5 µl per well of a 24-well plate. See Tables 1 and 2 for recommended starting concentrations.
- **Suboptimal oligonucleotide concentration**
  Determine the optimal sDNA concentration by titrating from 50 nM up to 200 nM in a 24-well plate. Determine the optimal 2'OMeRNA concentration by titrating from 0.5 µM up to 20 µM in a 24-well plate. See Tables 1 and 2 for recommended starting concentrations.
- **Poor quality of transfecting oligonucleotide**
  Use purified oligos from a quality vendor. Degradation can be detected by electrophoresis on an acrylamide gel. Ensure that the sequence of oligonucleotide is correct for your gene of interest or functional assay.
- **Fetal calf serum present during *Trans*IT®-Oligo Reagent/oligonucleotide complex formation**
  Use serum-free medium when forming the complexes.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, the optimal cell density for each cell type should be determined in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **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

- ***Trans*IT®-Oligo Reagent/oligonucleotide complex mixture and cells were not mixed thoroughly after adding the complex**
  Mix thoroughly to evenly distribute the complexes to all of the 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 amount of *Trans*IT®-Oligo Reagent/oligonucleotide complex mixture was used in transfection**
  Reduce the amount of *Trans*IT®-Oligo Reagent/oligonucleotide complex mixture in the transfection. See Tables 1 and 2 for recommended starting concentrations.
- **Cell density was too low at time of transfection**
  Grow cells to a higher cell density and repeat the transfection.
- **Media change may be necessary**
  If incubating for 48 hours, it may be necessary to change the complete media 24 hours post-transfection.

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 endotoxin removal from DNA:*
MiraCLEAN® Endotoxin Removal Kit (Product #5900)

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

For determination of gene expression efficiency:
Beta-Gal Staining Kit (Product # MIR 2600)

Additional 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 one year from the date of purchase if stored and handled properly.
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