*Trans*IT®-Oligo Transfection Reagent

Protocol for MIR 2160, 2164, 2165, 2166

Mirus | The Transfection Experts

INTRODUCTION

*Trans*IT®-Oligo Transfection Reagent is a broad spectrum reagent that enables high efficiency oligonucleotide or oligoribonucleotide delivery in many cell types.
*Trans*IT-Oligo can be used to transfect different kinds of oligos, e.g. phosphodiester DNA, phosphothioate DNA (sDNA), phosphothioate RNA (sRNA), 2'OMe RNA, 2'OMe RNA/sDNA chimerics, and morpholino/DNA duplexes. *Trans*IT-Oligo provides all the attributes of the trusted *Trans*IT series of transfection reagents: high transfection efficiency, low toxicity, serum compatibility, simplicity of use and reproducibility. Transfections with *Trans*IT-Oligo do not require medium changes and can be carried out in serum-containing medium.

SPECIFICATIONS

Storage	Store tightly capped at 4˚C. ***Do not freeze.*** ***Before each use***, warm to room temperature and vortex gently.
Product guarantee	6 months from the date of purchase, when properly stored and handled.

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

MATERIALS

Materials Supplied

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

Product No.	Quantity
MIR 2164	1 × 0.4 ml
MIR 2160	1 × 1.0 ml
MIR 2165	5 × 1.0 ml
MIR 2166	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified oligo
- Serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Oligo Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency transfection of oligos into multiple cell types using *Trans*IT-Oligo Transfection Reagent. Please refer to **Table 1** on Page 3 for recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** Determine the best cell density for each cell type to maximize transfection efficiency. Passage the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach appropriate cell density (generally 50–70% confluence) at the time of transfection. If this confluence does not produce optimal results, test cell densities outside of the recommended range.
- **Volume of *Trans*IT-Oligo Reagent.** Each cell type responds differently to a given transfection reagent. As a starting point, test 3 µl of *Trans*IT-Oligo Reagent per well of a 24-well plate. Vary the concentration of *Trans*IT-Oligo Reagent from 2–5 µl per well of a 24-well plate to find the best volume for further use. Tables 1 and 2 provide recommended starting conditions based on cell culture vessel size.
- **Oligo concentration.** Oligos used for transfection should be highly pure, 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. As a starting point, use 2 µM 2'OMe RNA oligo. Please refer to Table 1on Page 3 for recommended starting conditions.

  The optimal final sDNA concentration for transfection should be within the range of 50–200 nM. As a starting point, use 100 nM sDNA oligo. Refer to Table 2 on Page 3 for recommended starting conditions.
- **Complex formation conditions.** Prepare *Trans*IT-Oligo Reagent: oligo 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**.** *Trans*IT-Oligo Reagent 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 8–48 hours post-transfection. Incubation times will vary according to the experiment being performed.

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

OLIGO TRANSFECTION PROTOCOL

The following procedure describes how to perform oligo transfection using *Trans*IT-Oligo Transfection Reagent in 24-well plates. The surface areas of other culture vessels are different and transfection must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, *Trans*IT-Oligo Reagent, oligo and complete culture medium based on the surface area of the cell culture vessel (please refer to **Tables 1 and 2** for starting conditions for 2'OMe RNA oligo and sDNA oligo transfection, respectively).

This protocol recommends using half the volume of complete growth media compared to traditional transfection protocols. This ensures maximum oligo transfection efficiency. If full culture volume is desired, double the volume of complete growth media and oligo used.

Table 1: Recommended starting conditions for 2'OMe RNA oligo transfection with *Trans*IT-Oligo 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 (half volume protocol)	44 µl	125 µl	0.25 ml	0.5 ml	1.25 ml	7.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
*Trans*IT-Oligo Reagent	0.5 µl	1.5 µl	3 µl	6 µl	15 µl	93 µl	118 µl
2'OMe RNA (100 µM stock) 2 µM final	1 µl	3 µl	6 µl	12 µl	30 µl	180 µl	432 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 are required to be pipetted, dilute the *Trans*IT-Oligo Reagent in 100% ethanol before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-Oligo Reagent and reuse.

Table 2: Recommended starting conditions for sDNA oligo transfection with *Trans*IT-Oligo 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 (half volume protocol)	44 µl	125 µl	0.25 ml	0.5 ml	1.25 ml	7.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
*Trans*IT-Oligo Reagent	0.5 µl	1.5 µl	3 µl	6 µl	15 µl	93 µl	118 µl
sDNA (10 µM stock) 100 nM final	0.5 µl	1.5 µl	3 µl	6 µl	15 µl	90 µl	216 µl

Transient oligo transfection protocol per well of a 24-well plate

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells using the following guidelines. Ideally cells should be 50–70% confluent prior to transfection.

   For adherent cells: Plate cells in 0.5 ml complete growth medium/well at an optimal concentration (generally 0.4–1.2 × 10^5 cells/well).

   For suspension cells: Plate cells in 0.25 ml of complete growth medium per well, at a density of 1.6–2 × 10^5 cells per well.
2. Incubate the cell cultures overnight.

B. Prepare *Trans*IT-Oligo Reagent:Oligo complexes (Immediately before transfection)

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

1. Warm *Trans*IT-Oligo Reagent to room temperature and vortex gently before using.
2. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 3 µl of *Trans*IT-Oligo Reagent. Pipet gently to mix completely. For further optimization of your cell type and oligo, test additional levels of the *Trans*IT-Oligo Transfection Reagent (please refer to “Before You Start” on Page 2).
4. Incubate at room temperature for 5–20 minutes.
5. ***For 2'OMe RNA oligo transfection***: Add 6 µl of a 100 µM oligo stock solution (2 µM final concentration per well).

   For sDNA oligo transfection: Add 3 µl of a 10 µM oligo stock solution (100 nM final concentration per well).

   Pipet gently to mix completely.
6. Incubate at room temperature for 5–20 minutes.

C. Distribute the complexes to cells in complete growth medium

1. ***For adherent cells:*** Adjust the volume in the well to 0.25 ml of complete growth medium by removing 0.25 ml (half) of the original plating medium. Reducing the volume of medium in each well substantially decreases the amount of oligo required per well.

   For suspension cells: It is not necessary to replace the complete growth medium with fresh medium.
2. Add the *Trans*IT-Oligo Reagent:oligo complexes (prepared in Step B) drop-wise to different areas of the wells.
3. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-Oligo Reagent:oligo complexes.
4. Incubate for 4–48 hours or as required. It is not necessary to replace the complete growth medium with fresh medium.
5. 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.

TROUBLESHOOTING GUIDE

Problem	Solution
POOR OLIGO TRANSFECTION EFFICIENCY	
*Trans*IT-Oligo Reagent was not mixed properly.	Warm *Trans*IT-Oligo to room temperature and vortex gently before each use.
Suboptimal amount of *Trans*IT-Oligo Reagent	For optimization, test three levels of *Trans*IT-Oligo Reagent, e.g. 1, 3, and 5 µl per well of a 24-well plate. It may be necessary to titrate outside of this range depending on the cell type.
Suboptimal oligo concentration	Determine the optimal oligo concentration by titrating from 0.5–5 µM 2'OMe RNA or 50–200 nM sDNA (final concentration per well). Refer to "Before You Start" on Page 4.
Denatured oligo	To dilute oligo, use the manufacturer's recommended buffer. Avoid oligo degradation by using DNase or RNase-free handling procedures and plastic ware. Degradation of large oligos can be detected on acrylamide gels.
Incorrect oligo Sequence	Ensure that the sequence of the oligo is correct for your functional assay.
Poor quality of oligo	Avoid oligo degradation by using DNase or RNase-free handling procedures and plastic ware. Degradation of large oligos can be detected on acrylamide gels.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-Oligo Reagent: oligo 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. The presence of polyanions e.g. dextran sulfate or heparin can inhibit transfection. Use transfection medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hours post transfection.
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g. 4–48 hours).
Precipitate formation during transfection complex formation	During complex formation, scale all reagents including serum-free medium, *Trans*IT-Oligo Reagent, and oligo according to Tables 1 or 2 on Page 3. Precipitation may be observed when excess oligo (> 20 µM) is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of oligo, increase the volume of serum-free medium during complex formation by two-fold.
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add transfection complexes drop-wise to the 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	*Trans*IT-Oligo efficiently transfects cells cultured in serum-free medium; however, toxicity may be higher if serum is not present. If toxicity is a problem, consider adding serum to the culture medium.
Medium change or addition may be necessary	If incubating for 48–72 hours, it may be necessary to change the complete medium 24 hours post-transfection. Alternatively, add additional complete medium 4–24 hours post-transfection.
Cell density not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this density to ensure reproducibility. For most cell types, 50–70% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on cell type.

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
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.

RELATED PRODUCTS

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- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
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- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT-PRO® Transfection Kit
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT-siQUEST® Transfection Reagent
- *Trans*IT-TKO® Transfection Reagent
- *Trans*IT®-siPAK Kit and *Trans*IT®-siPAK Plus Kit
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