*Trans*IT®-2020 Transfection Reagent

Protocol for Product Nos. MIR 5400, 5404, 5405, 5406

Mirus | The Transfection Experts

INTRODUCTION

*Trans*IT®-2020 is a broad spectrum transfection reagent that provides superior transfection of plasmid DNA into mammalian cells. *Trans*IT-2020 is suitable for both transient and stable transfection and works well in typically hard-to-transfect cell lines. *Trans*IT-2020 is comprised of animal-origin free components and is serum compatible, eliminating the need for any culture media change after transfection.

SPECIFICATIONS

Storage	Store *Trans*IT-2020 Reagent at –20°C. ***Before each use***, warm to room temperature and vortex gently.
Stability/ Guarantee	6 months from the date of purchase, when properly stored and handled.

Warm *Trans*IT-2020 to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

The *Trans*IT-2020 Transfection Reagent is supplied in ***one*** of the following formats.

Product No.	Quantity	Number of Transfections
MIR 5404	1 × 0.4 ml	Up to 160
MIR 5400	1 × 1.0 ml	Up to 400
MIR 5405	5 × 1.0 ml	Up to 2000
MIR 5406	10 × 1.0 ml	Up to 4000

One transfection reaction is defined per well of a 6-well plate using a 1:1 ratio of *Trans*IT-2020 to plasmid DNA.

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified DNA (e.g., plasmid or linear DNA)
- Serum-free medium (e.g., Opti-MEM®I)
- Sterile polystyrene 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 cell type to ensure successful transfections. The suggestions below generally yield highly efficient transfection. **Table 1** below presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** Determine the optimal 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 optimal cell density (generally 40–80% confluence) at the time of transfection.
- **DNA Purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preps that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are optimal. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.
- **Ratio of *Trans*IT®-2020 Reagent to DNA.** Determine the optimal *Trans*IT-2020 Reagent: DNA ratio for each cell type. Start with 3 µl of *Trans*IT-2020 Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-2020 Reagent from 1–4 µl per 1 µg DNA to find the optimal ratio. **Table 1** below provides recommended starting conditions based on cell culture vessel size.
- **Complex formation conditions.** Prepare *Trans*IT-2020 Reagent:DNA complexes in serum-free growth medium. Mirus recommends Opti-MEM®I Reduced Serum medium. Use DNA stocks that range in concentration from 1–3 µg/µl.
- **Cell Culture conditions:** Culture cells in the appropriate medium, with or without serum. There is no need to perform a media change to remove the transfection complexes.
- **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 (100X stock of penicillin/streptomycin diluted up to 0.1–1X final concentration).
- **Post-transfection incubation time.** Determine the optimal incubation time post-transfection for each cell type. Test a range of incubation times. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment and the nature of the plasmid used.

Do not use DNA prepared using miniprep kits for transfection.

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

Transfection complexes can be added directly to cells cultured in complete growth media containing serum and up to 0.1–1X antibiotics.

Table 1. Recommended starting conditions for DNA transfections with *Trans*IT-2020 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^3
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	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-2020 Reagent	0.28 µl	0.79 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 *Trans*IT-2020 volumes are required to be pipetted, dilute the *Trans*IT-2020 Reagent in 80% ethanol before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-2020 Reagent and reuse.

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®-2020 Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel (see **Table 1** on Page 2).

Transient plasmid DNA transfection protocol for cells in 6-well plates

A. Plate cells

1. Approximately 18–24 hours before transfection, plate cells in 2.5 ml complete growth medium per well in a 6-well plate. Ideally cells should be 40–80% confluent prior to transfection.

   For adherent cells: Plate cells at the optimal concentration (generally 2–6 × 10^5 cells/well).

   For suspension cells: Plate cells at a density of 8–10 × 10^5 cells/well.
2. Incubate the cell cultures overnight.

Divide cultured cells 18–24 hours before transfection such that the cells reach optimal cell density at the time of transfection.

B. Prepare *Trans*IT-2020 Reagent:DNA complex (Immediately before transfection)

1. Warm *Trans*IT-2020 Reagent to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM®I Reduced Serum medium in a sterile polystyrene tube.
3. Add 2.5µg plasmid DNA.
4. Pipet gently to mix completely.
5. Add 7.5µl *Trans*IT-2020 Reagent to the diluted DNA mixture.
6. Pipet gently to mix completely.
7. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

Warm *Trans*IT-2020 to room temperature and vortex gently before each use.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-2020 Reagent:DNA complexes (prepared in Step B) drop wise to different areas of the wells containing plated cells. It is not necessary to replace the complete growth medium with fresh medium.
2. Gently rock the culture vessel back and forth and from side to side to evenly distribute the *Trans*IT-2020 Reagent:DNA complexes.
3. Incubate for 24–72 hours.
4. Harvest cells and perform a functional assay as required.

*Trans*IT-2020 is a low-toxicity reagent. There is no need to change fresh culture medium after transfection, unless required by your cell type or culture conditions.

For generating stable cell transtectants, 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 for selection of desired stable cells.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
Cell density (% confluence) not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this optimal density to ensure reproducibility. For most cell types, 40–80% confluence at the time of transfection is recommended.
	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.
*Trans*IT®-2020 Reagent was not mixed properly.	Warm *Trans*IT-2020 to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-2020 Reagent:DNA ratio	Determine optimal *Trans*IT-2020 Reagent: DNA ratio for each cell type. Titrate the *Trans*IT-2020 Reagent from 1–4 µL per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Determine the DNA concentration accurately. 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 reagent accordingly.
	Very highly concentrated DNA may produce a precipitate during complex formation.
	If you do not observe any expression of your target insert, verify the sequence of the plasmid DNA to ascertain correct translation frame.
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 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.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT 2020 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 media containing serum and up to 0.1–1X antibiotics.
	Polyanions such as dextran sulfate or heparin can inhibit transfection. Use culture medium that does not contain these polyanions.
Transfection complexes were added to cells cultured in serum-free medium	Allow *Trans*IT-2020 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 media change is required.
Proper controls were not included	To verify efficient transfection, use *Trans*IT-2020 Reagent to deliver a reporter plasmid such as a luciferase, beta-gal or GFP encoding plasmid.
	To assess delivery efficiency of plasmid DNA, use Mirus' Label IT® Nucleic Acid Intracellular Localization or prelabeled *Label* IT® Plasmid Delivery Controls (please refer to Related Products on Page 6).

TROUBLESHOOTING GUIDE continued

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (for example, from 12–72 hours). The optimal incubation time is generally 24–48 hours.
Cell morphology has changed	A high or low cell passage number can reduce transfection efficiency. Maintain a similar passage number between experiments to ensure reproducibility. **Do not** use cells with very high passage numbers as they tend to become refractory to transfection.
HIGH CELLULAR TOXICITY	
Cell density not optimal at time of transfection	Determine optimal cell density for each cell type to maximize transfection efficiency. Use this optimal density to ensure reproducibility. For most cell types, 40–80% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on cell type.
*Trans*IT-2020 transfection complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-2020 Reagent transfection complex 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.
*Trans*IT-2020 Reagent: nucleic acid complexes added to cells cultured in serum-free medium	*Trans*IT-2020 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.
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.
Expressed target gene is toxic to cells	Include a control with empty vector and TransIT-2020 to compare 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 and using carrier DNA such as an empty cloning vector to maintain the *Trans*IT-2020:DNA ratio.
Cell morphology has changed	A high or low cell passage number can make cells more sensitive to transfection reagents. Maintain a similar passage number between experiments to ensure reproducibility.

RELATED PRODUCTS

- MiraCLEAN® Endotoxin Removal Kits
- *Label* IT® Tracker Nucleic Acid Intracellular Localization Kits
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- *Trans*IT®-*InVivo* Gene Delivery Kits
- *Trans*IT®-QR and *Trans*IT®-EE Delivery Solutions and Kits
- *Label* IT® Plasmid Delivery Controls

For details on the above mentioned products, visit www.mirusbio.com.

Contact Mirus Bio for additional information.

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For publications citing the use of *Trans*IT® series of transfection reagents, visit www.mirusbio.com.