*Trans*IT-PRO® Transfection Kit

Protocol for MIR 5700 and 5760

Mirus The Transfection Experts

INTRODUCTION

*Trans*IT-PRO® Transfection Kit consists of a DNA transfection reagent and boost combination specifically developed for mammalian protein production in suspension 293 and CHO derived cells. The PRO Boost Reagent is optional and enhances expression in certain media formulations. *Trans*IT-PRO Transfection Reagent and PRO Boost Reagent are comprised of animal-origin free components that are compatible with many chemically defined media formulations. Use of *Trans*IT-PRO Transfection Kit eliminates the need for a culture medium change post-transfection and is suitable for both transient and stable transfection.

SPECIFICATIONS

Storage	Store *Trans*IT-PRO Transfection Reagent and PRO Boost Reagent at –20°C. ***Before each use***, warm to room temperature and vortex gently.
Stability/ Guarantee	1 year from the date of purchase, when properly stored and handled.

CAUTION: Standard safe laboratory practices should be maintained when using all chemical transfection reagents. ***Please refer to product MSDS for full safety precautions.***

MATERIALS

Materials supplied

*Trans*IT-PRO Transfection Kit is supplied in ***one*** of the following formats. Please inquire about a custom quote for bulk quantities.

Product No.	Volume of *Trans*IT-PRO Reagent	Volume of PRO Boost Reagent
MIR 5700	1 × 1.0 ml	1 × 1.5 ml
MIR 5760	1 × 10 ml	1 × 15 ml

Materials required, but not supplied

- Protein production cell line
- Culture vessels (e.g. Corning® Cat. No. 431143 or 4500-125, Whatman® Cat. No. 7701-5110 and sealer Qiagen® Cat. No. 19570)
- Appropriate cell culture medium
- Purified DNA
- Serum-free medium (e.g., OptiPRO™ SFM, Invitrogen Cat. No. 12309)
- Sterile tube for transfection complex preparation
- Micropipets
- Orbital shaker (e.g. New Brunswick Innova 2000) or platform stirrer (e.g. Thermolyne Cellgro stirrer 45600)
- Reporter assay, as required

New to transfection? Use the QR code below for a video highlighting recommended tips and suggestions.

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfection. The suggestions below generally yield high efficiency transfection using *Trans*IT-PRO Transfection Kit. Please refer to the **Table 1** on page 3 for recommended starting conditions depending on culture vessel size.

- **Cell density at transfection.** Determine the optimal cell density for each cell type to maximize transfection efficiency. Typically, a cell density of 0.5–1.0 × 10^6 cells/ml is desired at the time of transfection. Cells should be actively dividing at the time of transfection. Ideally, cells should be maintained 18–24 hours prior to transfection and seeded into culture flask immediately prior to transfection.
- **DNA concentration**. Determine the optimal DNA concentration for each cell type. Start with 1µg of DNA per ml of culture. Vary the concentration of DNA from 0.5–2.0 µg/ml to find the best working DNA concentration.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are desired. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.
- **Ratio of *Trans*IT-PRO Reagent to DNA.** Determine the best *Trans*IT-PRO Reagent:DNA ratio for each cell type. Start with 1 µl of *Trans*IT-PRO Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-PRO Reagent from 0.5–2 µl per 1 µg DNA to find the optimal ratio.
- **Ratio of PRO Boost Reagent to DNA.** The use of PRO Boost Reagent for transfection is optional and enhances expression in certain media formulations. When using PRO Boost Reagent, determine the best PRO Boost Reagent:DNA ratio for each cell type. Start with 0.5 µl of PRO Boost Reagent per 1 µg of DNA. Vary the concentration of Boost Reagent from 0–1.5 µl per 1 µg DNA to find the optimal ratio (e.g. 0, 0.5, 1 and 1.5 µl per 1 µg DNA). **Do not** use the PRO Boost Reagent alone for transfection.
- **Cell culture conditions:** Culture cells in the appropriate medium, temperature and CO_2 concentration. For suspension CHO cells, Mirus recommends BD Select™ CD1000 Medium (Cat. No. 215204) supplemented with L-glutamine (4 mM final concentration) or GIBCO® Freestyle™ CHO Expression Medium (Cat. No. 12651) supplemented with L-glutamine (4 mM final concentration). For suspension 293 cells, Mirus recommends GIBCO® Freestyle™ 293 Expression Medium (Cat. No.12338). Alternative media formulations may also be compatible with transfection. Medium formulation can have profound effects on the transfection efficiency; in particular, Pluronic® surfactant greater than 0.1 g/l (0.01% w/v) can inhibit transfection efficiencies. There is no need to perform a medium change to remove the transfection complexes. Medium additives or supplements can be added 24 hours post-transfection.
- **Complex formation conditions.** Prepare *Trans*IT-PRO Reagent:PRO Boost:DNA complexes in serum-free medium without additional supplements. Mirus recommends OptiPRO™ SFM medium for complex formation.
- **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 culture medium containing low levels of antibiotics (100X stock of penicillin/streptomycin diluted up to 0.1–1X final concentration).

Do not use DNA prepared using miniprep kits for transfection.

Do not add surfactants such as Pluronic at levels higher than 0.01% w/v during transfection.

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

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

- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time will vary depending on the goal of the experiment and the nature of the plasmid used; a general guideline is 2–5 days post-transfection. For secreted antibody constructs, optimal titers are typically obtained 5–7 days post-transfection in batch fermentation.

Table 1. Calculation worksheet for scaling of reagents for DNA transfection with *Trans*IT-PRO® Transfection Kit.

Starting conditions per milliliter of complete growth medium						
	Per 1 ml			Total culture volume		Reagent quantities
Complex medium (e.g. OptiPRO™)	0.1	ml	×	_____ml	=	_____ml
Plasmid DNA (1µg/µl stock)	1	µl	×	_____ml	=	_____µl
*Trans*IT-PRO Reagent	1	µl	×	_____ml	=	_____µl
PRO Boost Reagent (optional)	0.5	µl	×	_____ml	=	_____µl

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections in 125 ml Erlenmeyer shake flasks using 20 ml of complete growth medium. The medium and reagent volume requirements of other culture dishes and flasks are different and must be scaled accordingly. Appropriately increase or decrease the amounts of complex medium, *Trans*IT-PRO Transfection Reagent, PRO Boost Reagent, DNA and complete culture medium based on the **volume of complete growth medium** used in the cell culture vessel. To calculate the required reagent quantities based on the recommended starting conditions and total culture volume, please refer to the calculation worksheet in **Table 1**.

Transient plasmid DNA transfection protocol for cells in 125 ml Erlenmeyer shake flask (20 ml culture volume)

A. Maintenance of cells

1. Maintain cells 18–24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection.
2. Incubate cells overnight at appropriate temperature and CO_2 levels (e.g. 37°C, 5–8% CO_2).

Divide cultured cells 18–24 hours before transfection to ensure that cells are actively dividing at the time of transfection.

B. Prepare *Trans*IT-PRO Reagent:PRO Boost:DNA complexes (Immediately before transfection)

1. Just prior to transfection, seed cells at a density of 0.5–1.0 × 10^6 cells/ml into transfection culture vessel (e.g. 20 ml per 125 ml Erlenmeyer shake flask). In general, a higher density of cells will increase protein yield per volume.
2. Warm *Trans*IT-PRO and PRO Boost Reagents to room temperature and vortex gently before using.
3. Place 2 ml of OptiPRO™ SFM in a sterile tube.
4. Add 20 µg plasmid DNA (20 µl of a 1 ug/µl stock). Pipet gently to mix completely.
5. Add 20 µl *Trans*IT-PRO Reagent to the diluted DNA solution. Pipet gently to mix completely.

Warm *Trans*IT-PRO and PRO Boost Reagents to room temperature and vortex gently before each use.

6. **(Optional)** Add 10 µl PRO Boost Reagent to the diluted *Trans*IT-PRO Reagent:DNA solution. Pipet gently to mix completely.
7. Incubate at room temperature for 10-30 minutes to allow sufficient time for complexes to form.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-PRO Reagent:PRO Boost:DNA complexes (prepared in Step B) to culture vessel.
2. Shake flasks on an orbital shaker (120 rpm when using a shaker with a 2 cm orbital throw) at appropriate temperature and CO_2 levels (e.g. 37°C, 5–8% CO_2).
3. Incubate for 2–5 days, depending on the nature of the protein and detection method. For secreted antibody constructs, optimal titers are typically obtained 5–7 days post-transfection in batch fermentation.
4. Harvest cells and/or supernatant and assay as required.

There is no need to change culture medium after transfection, unless required by your cell type or culture conditions.

TROUBLESHOOTING GUIDE

New to transfection?
Use the QR code to the right for a video highlighting recommended tips and suggestions.

LOW PLASMID DNA TRANSFECTION EFFICIENCY

Problem	Solution
Medium formulation incompatible with transfection	Transfection complexes must be formed in serum-free medium without any additional supplements. Mirus recommends OptiPRO™ SFM medium for complex formation.
	Complete growth medium formulation has profound impacts on transfection efficiencies. To test if medium formulation is adversely affecting transfection, try a 50%:50% mix with a compatible medium or adapt cells to a compatible medium formulation. For suspension CHO cells, Mirus recommends BD Select™ CD1000 Medium (Cat. No. 215204) supplemented with L-glutamine (4 mM final concentration) or GIBCO® Freestyle™ CHO Expression Medium (Cat. No. 12651) supplemented with L-glutamine (4 mM final concentration). For suspension 293 cells, Mirus recommends GIBCO Freestyle 293 Expression Medium (Cat. No. 12338). Other media formulations may also be compatible with transfection.
	If the cells do not readily adapt to a compatible medium, try a stepwise sequential adaptation protocol according to the media manufacturer's instructions. As a general guideline, seed cells at a density of 3.0–5.0 × 10^5 cells/ml in a mix of 75% current and 25% compatible media for 2–4 passages until the cells return to normal doubling time and viability is > 80%. Do not passage cells if viability is below 80%. Increase the ratio of compatible media (e.g. 50% current and 50% compatible media) stepwise monitoring doubling and viability as outlined above until 100% compatible media is reached. Create a new cell bank in freezing medium (10% DMSO and 90% compatible medium).
Complete growth medium volume too high based on culture vessel size	For shake flasks, we recommend that the complete growth medium does not exceed one-third the capacity of the flask (e.g. ≤ 40 ml in a 125 ml Erlenmeyer flask).
	For spinner flasks, the maximum complete growth medium is equivalent to the capacity of the flask although cell movement and aeration will vary depending on the culture volume.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-PRO Reagent:PRO Boost:DNA complexes in serum-free growth medium. We recommend OptiPRO SFM for complex formation. Once transfection complexes are formed, they can be added directly to cells cultured in complete growth medium +/- serum and/or 0.1–1X antibiotics.
	Polyanions such as dextran sulfate or heparin can inhibit transfection. Use culture medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hours post transfection.
	Pluronic surfactant and other unidentified components of commercially available medium formulations can inhibit transfection. Avoid growth medium containing levels of Pluronic surfactant greater than 0.1 g/l (0.01% w/v) during transfection. If necessary, higher levels of Pluronic surfactant can be added 24 hours post transfection.
Cells not actively dividing at the time of transfection	Divide the culture 18–24 hours before transfection to ensure that the cells are actively dividing at time of transfection. Determine the optimal cell density for each cell type to maximize transfection efficiency. Typically, a cell density of 0.5–1.0 × 10^6 cells/ml at the time of transfection is desired. Ideally, cells should be maintained 18–24 hours prior to transfection and seeded into culture flask immediately prior to transfection. Cells may be maintained normally after transfection.

TROUBLESHOOTING GUIDE continued

LOW PLASMID TRANSFECTION EFFICIENCY

Problem	Solution
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 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 as it might contain high levels of endotoxin.
*Trans*IT-PRO and PRO Boost Reagents were not mixed properly	Warm *Trans*IT-PRO and PRO Boost Reagents to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-PRO Reagent:DNA ratio	Determine the best *Trans*IT-PRO Reagent:DNA ratio for each cell type. Titrate the *Trans*IT-PRO Reagent from 0.5–2 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal PRO Boost Reagent:DNA ratio	Determine the best PRO Boost Reagent:DNA ratio for each cell type. Titrate the PRO Boost Reagent from 0–1.5 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Confirm DNA concentration and purity. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8–2.0.
	The optimal DNA concentration generally ranges between 0.5–2.0 µg/ml of culture medium. Start with a DNA concentration of 1 µg/ml. Consider testing more or less DNA while scaling the amount of *Trans*IT-PRO Transfection Reagent accordingly.
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Transfection incubation time	Determine the optimal transfection incubation time for each cell type and experiment. The optimal incubation time is generally 2–5 days, but will vary depending on the goal of the experiment and the nature of the plasmid used. For secreted antibody constructs, optimal titers are typically obtained 5–7 days post-transfection in batch fermentation.
Precipitate formation during transfection complex formation	Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. As recommended in the protocol, always dilute DNA first in serum-free complex formation medium and mix before adding *Trans*IT-PRO and PRO Boost Reagent (optional) to the diluted DNA mixture during complex formation.
Proper experimental controls were not included	To verify efficient transfection, use *Trans*IT-PRO Transfection Kit to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.
	To assess delivery efficiency of plasmid DNA, use Mirus *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid or Mirus prelabeled *Label* IT Plasmid Delivery Controls (please refer to Related Products on Page 8).

TROUBLESHOOTING GUIDE continued

HIGH CELLULAR TOXICITY	
Problem	**Solution**
Shake/ spin culture conditions not optimal	Excessive agitation is harmful to cells. Monitor viability of cells using trypan blue exclusion.
Cells not properly adapted to growth culture medium prior to transfection	Check the viability of cultured cells before transfection. Ensure complete adaptation to growth culture medium by verifying consistent doubling times and viability ≥ 90% using trypan blue exclusion.
Cell density not optimal at time of transfection	Determine the best cell density for each cell type to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most suspension CHO and 293 derived cell types, a cell density of 0.5–1.0 × 10^6 cells/ml is recommended at the time of transfection, but use of higher or lower densities may be desirable depending on cell type, length of experiment and feeding schedule.
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/or refractory to transfection. Maintain a similar passage number between experiments to ensure reproducibility.
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 as it might contain high levels of endotoxin.
Expressed target gene is toxic to cells	Compare toxicity levels against a cells alone control and cells transfected with an empty vector to assess 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. Maintain the optimal *Trans*IT-PRO Reagent:DNA ratio by using carrier DNA such as an empty cloning vector.

Technical Support | Toll-free (U.S.) 888.530.0801 | Direct: 608.441.2852 | techsupport@mirusbio.com | www.mirusbio.com

RELATED PRODUCTS

- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- *Trans*IT®-3D Transfection Reagent
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT®-QR and *Trans*IT®-EE Delivery Solutions and Kits
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.TheTransfectionExperts.com/reagentagent

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

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