

TransIT-PRO[®] Transfection Kit

Protocol for MIR 5700 and 5760

Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/5700



INTRODUCTION

TransIT-PRO[®] Transfection Kit was specifically developed for mammalian protein production in suspension CHO and 293 cell types. TransIT-PRO[®] Transfection Kit is comprised of TransIT-PRO[®] Transfection Reagent and the PRO Boost Reagent, which may enhance expression up to two-fold in certain media formulations. Both components are animal-origin free and compatible with many chemically defined media formulations. Use of TransIT-PRO[®] Transfection Kit eliminates the need for a culture medium change post-transfection and is suitable for both transient and stable transfection.

TransIT-PRO[®] Transfection Reagent is a key component of the CHOgro[®] Expression System (MIR 6260) and CHOgro[®] High Yield Expression System (MIR 6270), which are optimized platforms for transient, high titer protein production in suspension CHO derived cells. The complete CHOgro[®] systems contain CHOgro[®] Expression Medium, TransIT-PRO[®] Transfection Reagent, CHOgro[®] Complex Formation Solution and media supplements L-Glutamine and Poloxamer 188.



For more information on the CHOgro[®] Expression Systems, please visit: mirusbio.com/CHOgro

SPECIFICATIONS

Storage	Store TransIT-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 SDS for full safety precautions.*

MATERIALS

Materials Supplied

TransIT-PRO[®] Transfection Kit is supplied in one of the following formats. Please inquire about a custom quote for bulk quantities:

Product No.	Volume of TransIT-PRO [®] Reagent	Volume of PRO Boost Reagent
MIR 5700	1 × 1 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; Thomson Cat. No. 931110)
- Appropriate cell culture medium
- Purified, endotoxin-free DNA
- Serum-free medium (e.g. Opti-MEM[®], Invitrogen Cat. No. 31985; OptiPRO[™] SFM, Invitrogen Cat. No. 12309; CHOgro[®] Complex Formation Solution, Mirus Cat. No. MIR 6210)
- Sterile tube for transfection complex preparation
- Orbital shaker (e.g. New Brunswick Innova 2000) or platform stirrer (Thermolyne Cellgro Stirrer 4500)
- Reporter assay as required

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 *TransIT-PRO*® Transfection Kit. Please refer to **Table 1** on Page 3 for recommended starting conditions depending on culture vessel size.

- **Cell density at transfection.** Determine the optimal density for each cell type to maximize transfection efficiency. Ideally, cells should be passaged 18-24 hours prior to transfection to obtain a next day density of $4 - 10 \times 10^6$ cells/ml. This allows for a two to five-fold dilution with complete medium immediately prior to transfection for a final density of 2×10^6 cells/ml at the time of transfection. Cultures should be placed at 37°C, 8% CO₂ prior to transfection.
- **DNA concentration.** Determine the optimal DNA concentration for each cell type. Start with 1 µg of DNA per ml of culture. Vary the concentration of DNA from 0.5-2 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 an A260/A280 absorbance ratio of 1.8-2.0 are desirable. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.
- **Ratio of *TransIT-PRO*® Reagent to DNA.** Determine the best *TransIT-PRO*® Reagent:DNA ratio for each cell type. Start with 1 µl of *TransIT-PRO*® Reagent per 1 µg of DNA. Vary the concentration of *TransIT-PRO*® Reagent from 0.5-2 µl per 1 µg of DNA to find the optimal ratio.
- **Ratio of PRO Boost Reagent to DNA.** The PRO Boost Reagent is optional and may enhance expression up to two-fold in certain media formulations (e.g. Freestyle™ CHO Expression Medium). 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 PRO Boost Reagent from 0-1.5 µl per 1 µg of DNA to find the optimal ratio. **Do not** use PRO Boost Reagent alone for transfection.
- **Cell culture conditions.** Culture cells in the appropriate medium at 37°C in 8% CO₂. For suspension CHO cells, we recommend CHOgro® Expression Medium (MIR 6200) supplemented with L-Glutamine (4 mM final concentration, MIR 6240) and Poloxamer 188 (0.3% final concentration, MIR 6230). For suspension 293 cells, we recommend Gibco™ Freestyle™ F17 Expression Medium (Cat. No. A13835). Additional medium additives or supplements can be added 24 hours post-transfection. Alternative media formulations may also be compatible with transfection.
- **Complex formation conditions.** Prepare *TransIT-PRO*® Reagent:PRO Boost:DNA complexes in serum-free medium without additional supplements (e.g. Opti-MEM®, OptiPRO™ SFM or CHOgro® Complex Formation Solution).
- **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).
- **Post-transfection incubation time.** The optimal incubation time will vary depending on cell type, experimental goal and the nature of the plasmid used. For general protein constructs, maximum titers can be obtained as soon as 2-5 days post-transfection. For secreted antibody constructs, maximum titers are typically obtained 5-7 days post-transfection when cells are grown by batch fermentation and maintained at 37°C. For suspension CHO cells cultured with the CHOgro® Expression Systems, optimal titers are obtained at 32°C, 7-14 days post-transfection in batch culture.



Do not use DNA prepared using miniprep kits which result in high levels of residual endotoxin.



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.

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. Increase or decrease the amounts of serum-free complex formation medium, *TransIT-PRO*® Transfection Reagent, PRO Boost Reagent (optional) and DNA based on the **volume of complete growth medium** used in alternate cell culture vessels. Refer to the scaling worksheet in **Table 1**.

Table 1. Transfection scaling worksheet for *TransIT-PRO*® Transfection Kit

Starting conditions per milliliter of complete growth medium					
	Per 1 ml		Total culture volume		Reagent quantities
Complex medium (e.g. Opti-MEM®)	0.1	ml	×	_____ ml	= _____ ml
Plasmid DNA (1 µg/µl stock)	1	µl	×	_____ ml	= _____ µl
<i>TransIT-PRO</i> ® Transfection Reagent	1	µl	×	_____ ml	= _____ µl
PRO Boost Reagent (optional)	0.5	µl	×	_____ ml	= _____ µl

Transient Plasmid DNA Transfection Protocol for Cells in 125 ml Erlenmeyer Shake Flask (20 ml Culture Volume)

A. Maintenance of cells

1. Passage cells 18-24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection.
NOTE: Perform cell counts and evaluate viability daily to ensure that cells are doubling every 24 hours and ≥ 95% viable by trypan blue exclusion. Do NOT proceed with transfection if cells are not doubling normally or are < 95% viable.
2. Incubate cells overnight at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂).



Passage cells 18-24 hours before transfection to ensure that cells are actively dividing at the time of transfection.

B. Prepare *TransIT-PRO*® Reagent:PRO Boost:DNA complexes

1. Immediately prior to transfection, seed cells at a density of 2×10^6 cells/ml into a transfection culture vessel (e.g. 20 ml per 125 ml Erlenmeyer shake flask).
2. Warm *TransIT-PRO*® and PRO Boost Reagents to room temperature and vortex gently.
3. Place 2 ml of serum-free medium (e.g. Opti-MEM®) in a sterile tube.
4. Add 20 µg of plasmid DNA (20 µl of a 1 µg/µl stock). Pipet gently to mix.
5. Add 20 µl of *TransIT-PRO*® Reagent to the diluted DNA solution. Pipet gently to mix.
6. (Optional) Add 10 µl of PRO Boost Reagent to the diluted *TransIT-PRO*® Reagent:DNA solution. Pipet gently to mix.
7. Incubate complexes at room temperature to allow sufficient time for complexes to form. For complexes added to suspension 293 cells, we recommend a 15-20 minute incubation. For complexes added to suspension CHO cells, we recommend a 5-10 minute incubation.



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

C. Distribute the complexes to cells in complete growth medium

1. Add transfection 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 CO₂ levels (e.g. 37°C, 5-8% CO₂).
3. The optimal incubation time will depend on cell type used, experimental goal and the nature of the plasmid used. For secreted antibody constructs, maximum titers are typically obtained 5-14 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

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 Opti-MEM® or OptiPRO™ SFM for complex formation. If using the CHOgro® Expression System, we recommend CHOgro® Complex Formation Solution.
	Complete growth medium formulation has profound effects 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 CHOgro® Expression Medium (MIR 6200) supplemented with L-Glutamine (4 mM final concentration, MIR 6240) and Poloxamer 188 (0.3% final concentration, MIR 6230). For suspension 293 cells, Mirus recommends Gibco Freestyle™ F17 Expression Medium (Cat. No. A13835). 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 $3 - 5 \times 10^5$ 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 or as specified by the manufacturer).
	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 <i>TransIT-PRO</i> ® Reagent:PRO Boost:DNA complexes in serum-free growth medium. We recommend Opti-MEM® for complex formation. If using the CHOgro® Expression System, we recommend CHOgro® Complex Formation Solution. 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.
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. Perform cell counts and evaluate viability daily to ensure that cells are doubling every 24 hours and $\geq 95\%$ viable by trypan blue exclusion. Do NOT proceed with transfection if cells are not doubling normally or are < 95% viable.

TROUBLESHOOTING GUIDE continued

LOW PLASMID DNA 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.
<i>TransIT-PRO</i> ® and PRO Boost Reagents were not mixed properly	Warm <i>TransIT-PRO</i> ® and PRO Boost Reagents to room temperature and vortex gently before each use.
Suboptimal <i>TransIT-PRO</i> ® Reagent:DNA ratio	Determine the best <i>TransIT-PRO</i> ® Reagent:DNA ratio for each cell type. Titrate the <i>TransIT-PRO</i> ® Reagent from 0-2 µl per 1 µg of 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 of DNA. Refer to “Before You Start” on Page 2.
Suboptimal DNA concentration	Confirm DNA concentration and purity. Use plasmid DNA preps that have an A260/A280 absorbance ratio of 1.8-2.0.
	The optimal DNA concentration generally ranges between 0.5-2 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 <i>TransIT-PRO</i> ® Transfection Reagent accordingly.
Incorrect vector sequence	If no expression of your target is observed, verify the sequence of the plasmid DNA.
Transfection incubation time	The optimal incubation time will vary depending on the cell type, experimental goal and the nature of the plasmid used. For general protein constructs, maximum titers can be obtained as soon as 2-5 days post-transfection. For secreted antibody constructs, maximum titers are typically obtained 5-7 days post-transfection when cells are grown by batch fermentation and maintained at 37°C. For suspension CHO cells cultured with the CHOgro® Expression System and moved from 37°C to 32°C hours post-transfection, 10-14 day incubations are recommended.
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 <i>TransIT-PRO</i> ® and PRO Boost Reagent (optional) to the diluted DNA mixture during complex formation.
Proper experimental controls were not included	To verify efficient transfection, use <i>TransIT-PRO</i> ® Transfection Kit to deliver a positive control such as luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid. For secreted antibody constructs, we recommend verifying expression with the Human IgG1 Expression Control (MIR 6250).
	To assess delivery efficiency of plasmid DNA, use Mirus <i>Label IT</i> ® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid or Mirus prelabeled <i>Label IT</i> ® Plasmid Delivery Controls (please refer to Related Products on Page 7).

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 are $\geq 95\%$ 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 and cell growth post-transfection. Use this cell density in subsequent experiments to ensure reproducibility. For most suspension CHO and 293 derived cell types, a cell density of 2×10^6 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 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 TransIT-PRO® Reagent:DNA ratio by using carrier DNA such as an empty cloning vector.

RELATED PRODUCTS

- CHOgro® Expression System
- CHOgro® High Yield Expression System
- CHOgro® Transfection and Titer Enhancer Kit
- CHOgro® Expression Medium
- Human IgG1 Expression Control
- *TransIT-PRO®* Transfection Reagent
- *Label IT®* Plasmid Delivery Controls
- *Label IT®* Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- Ingenio® Electroporation Solution and Kits
- *TransIT-X2®* Transfection Reagent
- *TransIT®-2020* Transfection Reagent
- *TransIT®-LT1* Transfection Reagent

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



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.mirusbio.com/ra