# *Trans*IT-PRO® Transfection Reagent

Protocol for MIR 5720, 5730, 5740 and 5750

*Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/5740*

## INTRODUCTION

*Trans*IT-PRO® Transfection Reagent was developed by empirically testing proprietary lipid and polymer libraries for high transfection performance in suspension CHO and HEK 293 cell types. This reagent is animal-origin free and compatible with many chemically defined media formulations. Use of *Trans*IT-PRO® Transfection Reagent eliminates the need for a culture medium change post-transfection and is suitable for both transient transfection and stable cell line generation.

*Trans*IT-PRO® Transfection Reagent is a key component of the CHOgro® Expression System (MIR 6260), which is an optimized platform for transient, high titer protein production in suspension CHO derived cells. The complete CHOgro® Expression System contains CHOgro® Expression Medium, *Trans*IT-PRO® Transfection Reagent, CHOgro® Complex Formation Solution, and media supplements L-Glutamine and Poloxamer 188.

For more information on the CHOgro® Expression System, please visit the product page at www.mirusbio.com/CHOgro.

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

## SPECIFICATIONS

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

## MATERIALS

### Materials supplied

*Trans*IT-PRO® Transfection Reagent is supplied in one of the following formats. For bulk quantities, please inquire about a custom quote.

| Product No. | Volume of *Trans*IT-PRO® Reagent |
|---|---|
| MIR 5740 | 1 × 1.0 ml |
| MIR 5750 | 1 × 10 ml |

### Materials required, but not supplied

- Protein production cell line
- Culture vessels (e.g. Corning® Cat. No. 431143 or 4500-125, Thompson Cat.No. 931110)
- Appropriate cell culture medium
- Purified, endotoxin-free DNA
- Serum-free medium (e.g. OptiMEM®, Invitrogen Cat. No.31985, OptiPRO™ SFM, Invitrogen Cat. No. 12309)*****
- Sterile tube for transfection complex preparation
- Orbital shaker (e.g. New Brunswick Innova 2000) or platform stirrer (e.g. Thermolyne Cellgro stirrer 45600)
- Reporter assay, as required

*****When using *Trans*IT-PRO® with the CHOgro® Expression System, form transfection complexes in CHOgro® Complex Formation Solution.

**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 Reagent. Please refer to the **Table 1**(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 x $10^6$ cells/ml. This allows for a 2 to 5-fold dilution with complete medium immediately prior to transfection for a final density of 2 x $10^6$ cells/ml at the time of transfection. Cultures should be placed at 37°C, 8% $CO_2$ 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.
- **Reagent to DNA ratio.** 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 1-2 µl per 1 µg DNA to find the optimal ratio.
- **Cell culture conditions.** Culture cells in the appropriate medium at 37°C in 8% $CO_2$. For suspension CHO cells, Mirus recommends CHOgro® Expression Medium (MIR 6200) supplemented with L-Glutamine (4mM 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). There is no need to perform a medium change to remove the transfection complexes. 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 *Trans*IT-PRO® Reagent:DNA complexes in serum-free medium without additional supplements. Mirus recommends OptiMEM® or OptiPRO™ SFM medium for complex formation. When using *Trans*IT-PRO® with the CHOgro® Expression System, form transfection complexes in 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 System and moved from 37°C to 32°C at 24 hours post-transfection, 10-14 day incubations are recommended.

***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 growth media +/- serum and up to 0.1–1X antibiotics.

# 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 medium, *Trans*IT-PRO® Transfection Reagent, DNA, and complete culture medium based on the **volume of complete growth medium** used in alternate 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**.

**Table 1.** Calculation worksheet for scaling *Trans*IT-PRO® Reagent transfections.

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

*When using *Trans*IT-PRO® with the CHOgro® Expression System, form transfection complexes in CHOgro® Complex Formation Solution.

## 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 and to obtain a next day density of 4-10 x $10^6$ cells/ml.

   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 $CO_2$ 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: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 *Trans*IT-PRO® reagent to room temperature and vortex gently before using.
3. Place 2 ml of serum-free medium (e.g. OptiMEM®)* 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.
6. Incubate complexes at room temperature to allow sufficient time for complexes to form. For complexes added to suspension 293 cells, we recommend a15-20 minute incubation. For complexes added to suspension CHO cells, we recommend a 5-10 minute incubation.

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

### C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-PRO®: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. 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 OptiMEM® or OptiPRO™ SFM medium for complex formation. When using *Trans*IT-PRO® with the CHOgro® Expression System, form transfection complexes in 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 (4mM 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 3.0–5.0 × $10^5$ 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 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 *Trans*IT-PRO® Reagent:DNA complexes in serum-free growth medium. We generally recommend OptiMEM® or OptiPRO™ SFM. When using *Trans*IT-PRO® with the CHOgro® Expression System, form transfection complexes in 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 ≥ 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 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.<br>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® Reagent was not mixed properly | Warm *Trans*IT-PRO® Reagent 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 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 no expression of your target insert is observed, verify the sequence of the plasmid DNA. |
| 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 System and moved from 37°C to 32°C at 24 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 *Trans*IT-PRO® 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. 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 *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 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 ≥ 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 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 $2 \times 10^6$ 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.<br>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.<br>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.<br>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.<br>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. |

# RELATED PRODUCTS

- CHOgro® Expression System
- CHOgro® Expression Medium
- Human IgG1 Expression Control
- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits

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

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