*Trans*IT-HeLaMONSTER® Transfection Kit

Protocol for MIR 2900, 2904, 2905, 2906

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/2900

INTRODUCTION

*Trans*IT-HeLaMONSTER® Transfection Kit is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in HeLa cells and cell types of associated lineage. *Trans*IT-HeLaMONSTER Transfection Kit contains two components, namely: *Trans*IT®-HeLa Reagent and the MONSTER Reagent. This kit 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. Transfection with this kit does not require medium changes and can be carried out in serum-containing medium. This kit is suitable for both transient and stable transfection.

SPECIFICATIONS

Storage	Store both *Trans*IT-HeLa Reagent and MONSTER Reagent at -20°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

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

MATERIALS

Materials Supplied

The *Trans*IT-HeLaMONSTER Transfection Kit is supplied in ***one*** of the following formats.

Product No.	Volume of *Trans*IT-HeLa Reagent	Volume of MONSTER Reagent
MIR 2904	1 × 0.4 ml	1 × 0.4 ml
MIR 2900	1 × 1 ml	1 × 1 ml
MIR 2905	5 × 1 ml	5 × 1 ml
MIR 2906	10 × 1 ml	10 × 1 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified DNA
- 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 DNA Transfection

Optimize reaction conditions for each HeLa cell subtype to ensure successful transfections. The suggestions below yield high efficiency DNA transfection using the *Trans*IT-HeLaMONSTER Transfection Kit. **Tables 1–2** on Pages 3–4 present recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for HeLa cell subtypes at transfection is ≥80% confluence. Determine the optimal cell density for each HeLa cell subtype in order to maximize transfection efficiency. Divide the cells 18–24 hours before transfection to ensure that the cells are actively dividing and reach the appropriate cell density 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 desirable. DNA prepared using miniprep kits is not recommended as it may contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove any traces of endotoxin from your DNA preparation.

Do not use DNA prepared using miniprep kits for transfection.

- ***Trans*IT-HeLa Reagent:DNA ratio.** As a starting point, use 3 µl of *Trans*IT-HeLa Reagent per 1 µg of DNA. The optimal *Trans*IT-HeLa Reagent to DNA ratio can be determined by titrating the reagent from 2–4 µl per 1 µg of DNA. Please refer to Tables 1–2 on Pages 3–4 for recommended starting conditions.
- **MONSTER Reagent:DNA ratio.** Different MONSTER Reagent amounts may be required depending on the cell culture and experimental conditions. The optimal MONSTER Reagent:DNA ratio should be determined by titrating the reagent from 0–5 µl per 1 µg of DNA. Please refer to **Tables 1–2** on Pages 3–4 for recommended starting conditions.
- **Complex formation conditions.** Prepare *Trans*IT-HeLa:MONSTER:DNA 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-HeLaMONSTER Transfection Kit yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change.

Do not use serum or antibiotics in the medium during transfection 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 complete culture medium containing low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24–72 hours, but will vary depending on the goal of the experiment, nature of the plasmid, and the half-life of the expressed protein.

DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform 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-HeLa Reagent, MONSTER Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel. **Table 1** presents recommended starting conditions depending on culture vessel size.

Optimal transfection efficiencies can be achieved using the following protocol. An alternate protocol (please refer to Page 4) is provided which uses a slightly modified procedure to apply diluted MONSTER Reagent directly to the cells. Choose the protocol that achieves the highest transfection efficiency.

Table 1. Recommended starting conditions for DNA transfections with the *Trans*IT-HeLaMONSTER Transfection Kit.

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	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	0.25 µl	0.5 µl	1 µl	2.5 µl	15.5 µl	19.7 µl
*Trans*IT-HeLa Reagent	0.3 µl	0.75 µl	1.5 µl	3 µl	7.5 µl	46.5 µl	59.1 µl
MONSTER Reagent*	0.2 µl	0.50 µl	1 µl	2 µl	5 µl	31 µl	39.4 µl

*Different MONSTER Reagent amounts may be required depending on the cell culture and experimental conditions. The optimal MONSTER Reagent:DNA ratio should be determined by titrating the reagent from 0–5 µl per 1 µg of DNA.

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 of kit reagents need to be pipetted, dilute the *Trans*IT-HeLa and MONSTER reagent in serum-free medium and water, respectively. ***Do not*** store diluted reagents.

Transient DNA transfection protocol per well of a 6-well plate

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 ≥80% confluent prior to transfection.
2. Incubate the cell cultures overnight.

Divide cultured cells 18–24 hours before transfection to ensure active cell division at the time of transfection.

B. Prepare *Trans*IT-HeLa:MONSTER:DNA complexes (Immediately before transfection)

1. Warm *Trans*IT-HeLa and MONSTER reagents to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 2.5 µg (2.5 µl of a 1 µg/µl stock) DNA. Pipet gently to mix completely.
4. Add 7.5 µl *Trans*IT-HeLa Reagent to the diluted DNA mixture. Pipet gently to mix completely.
5. Add 5 µl MONSTER Reagent to the diluted *Trans*IT-HeLa:DNA mixture. Pipet gently to mix completely.

Warm *Trans*IT-HeLa and MONSTER reagents to room temperature and vortex gently before each use.

6. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.

C. Distribute the complexes to cells in complete growth medium

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

There is no need to change culture medium after transfection. If required, perform a medium change at least 4 hours post-transfection.

For generating stable cell transfectants, passage the cells 48-72 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 selection of cells that have undergone stable integration of DNA.

Alternate transient DNA transfection protocol per well of a 6-well plate

The following *Trans*IT-HeLaMONSTER Transfection Kit protocol uses a slightly modified procedure where diluted MONSTER Reagent is applied directly to the cells plated in a 6-well plate. In some cases, this protocol might yield higher transfection efficiency compared to the standard protocol. 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-HeLa Reagent, MONSTER Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel. **Table 2** presents recommended starting conditions depending on culture vessel size.

Table 2. Recommended starting conditions for DNA transfections with the *Trans*IT-HeLaMONSTER Transfection Kit using the alternate protocol.

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	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	0.25 µl	0.5 µl	1 µl	2.5 µl	15.5 µl	19.7 µl
*Trans*IT-HeLa Reagent	0.3 µl	0.75 µl	1.5 µl	3 µl	7.5 µl	46.5 µl	59.1 µl
Diluted MONSTER Reagent (0.1X)	2.0 µl	5.0 µl	10 µl	20 µl	50 µl	310 µl	394 µl

If small volumes of kit reagents need to be pipetted, dilute the *Trans*IT-HeLa and MONSTER reagent in serum-free medium and water, respectively. ***Do not*** store diluted reagents.

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 ≥80% confluent (~1–3 × 10^5 cells/well) prior to transfection.
2. Incubate the cell cultures overnight.

Divide cultured cells 18–24 hours before transfection to ensure active cell division at the time of transfection.

B. Prepare *Trans*IT-HeLa:DNA complexes (Immediately before transfection)

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

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

C. Dilution of MONSTER Reagent (Immediately prior to or during *Trans*IT-HeLa:DNA complex formation)

1. Warm MONSTER Reagent to room temperature and vortex gently before using.
2. In a sterile tube, dilute 5 µl MONSTER Reagent 10-fold in 45 µl sterile (DNase and RNase-free) water. Mix well.
3. Immediately return the stock MONSTER Reagent to -20˚C after use.

D. Distribute the complexes to cells in complete growth medium

1. If necessary, remove the medium from the cells prepared in Step A and replace with 2.5 ml per well of fresh complete growth medium.
2. Add the *Trans*IT-HeLa:DNA complexes 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-HeLa:DNA complexes.
4. Add the diluted MONSTER Reagent prepared in Step C drop-wise directly to the cells.
5. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the diluted MONSTER Reagent.
6. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
7. Harvest cells and assay as required.

There is no need to change culture medium after transfection. If required, perform a medium change at least 4 hours post-transfection.

For generating stable cell transfectants, passage the cells 48-72 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 selection of cells that have undergone stable integration of DNA.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW DNA TRANSFECTION EFFICIENCY	
*Trans*IT-HeLa or MONSTER Reagent was not mixed properly.	Warm *Trans*IT-HeLa and MONSTER Reagents to room temperature and vortex gently before each use.
Suboptimal amount of *Trans*IT-HeLa Reagent	Determine optimal amount of *Trans*IT-HeLa Reagent for each HeLa cell subtype. Titrate the *Trans*IT-HeLa Reagent from 2–4 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal amount of MONSTER Reagent	Determine optimal amount of MONSTER Reagent for each HeLa cell subtype. Titrate the MONSTER Reagent from 0–5 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 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 *Trans*IT-HeLa and MONSTER Reagents accordingly.
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 may contain high levels of endotoxin.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare transfection 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. 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.
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. Test a range of incubation times (e.g.12–72 hours). The best incubation time is generally 24–48 hours.
Cells not actively dividing at the time of transfection	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.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents including serum-free medium, *Trans*IT-HeLa Reagent, MONSTER Reagent and plasmid DNA according to Table 1–2 on Pages 3–4. Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold.

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TROUBLESHOOTING GUIDE continued

Problem	Solution
LOW DNA TRANSFECTION EFFICIENCY	
Proper experimental controls were not included	To verify efficient transfection, use *Trans*IT-HeLaMONSTER 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).
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add transfection complexes drop-wise to different areas of the wells containing plated 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	Allow transfection 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 medium change is required after the addition of transfection complexes to cells.
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 may 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-HeLaMONSTER:DNA ratio by using carrier DNA such as an empty cloning vector.
Cell density not optimal at time of transfection	Determine the best cell density for each HeLa cell subtype to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most HeLa cell subtypes, ≥80% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on cell type.
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

- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- Cell Culture Antibiotic Solutions
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT-PRO® Transfection Kit
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits

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