*Trans*IT®-HeLaMONSTER® Transfection Kit

Product Name	Volume of *Trans*IT®-HeLa Reagent	Volume of MONSTER Reagent	Product No.
*Trans*IT®-HeLaMONSTER® Transfection Kit	0.4 ml	0.4 ml	MIR 2904
	1 ml	1 ml	MIR 2900
	5 ml (5 × 1 ml)	5 ml (5 × 1 ml)	MIR 2905
	10 ml (10 × 1 ml)	10 ml (10 × 1 ml)	MIR 2906

Each milliliter of *Trans*IT®-HeLaMONSTER® Transfection Kit (MIR 2900) provides sufficient amounts of both reagents to perform up to 500 transfections in 6-well plates.

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT®-HeLaMONSTER® Transfection Kit was developed by the nucleic acid delivery specialists at Mirus Bio Corporation. This novel kit was specifically optimized to provide superior transfection efficiency in HeLa cells without sacrificing cellular health. HeLa cells have been inherently difficult to transfect yet have remained a prevalent cell line in the biological research field. The specificity of the *Trans*IT®-HeLaMONSTER® Transfection Kit makes this product a desirable alternative to broad spectrum transfection reagents. The kit provides all the attributes of the trusted *Trans*IT® Reagent line: high efficiency, low toxicity, simplicity of use and reproducibility. In addition, transfections with the *Trans*IT® Reagents do not require media changes and can be carried out in serum-containing media. The *Trans*IT®-HeLaMONSTER® Transfection Kit is quality control tested on ATCC HeLa cells. These significant features establish the *Trans*IT®-HeLaMONSTER® Transfection Kit as the product of choice for transfecting HeLa cells.

1.2 Specifications

Concentration: *Trans*IT®-HeLa Reagent: 1.33 mg/ml in 80% ethanol
MONSTER Reagent: 1 mg/ml in water

Storage: Store both reagents at -20˚C. Prior to use, warm the *Trans*IT®-HeLa Reagent and MONSTER Reagent to room temperature and gently vortex to dissolve any precipitate that may have formed.

Stability: 1 year when stored at -20˚C.

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of transfection parameters. The transfection protocols described in Sections 2.2 and 2.3 should result in efficient transfection of most HeLa cell subtypes; however, to ensure optimal results consider the following variables:

A. Media conditions - The *Trans*IT® - HeLaMONSTER® Kit yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection media change.

B. Cell density (% confluence) at the time of transfection - The recommended cell density for most HeLa cell types at transfection is 50-70% confluence. Determine the optimal cell density for each HeLa cell subtype in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.

C. DNA purity and concentration for transfection - DNA used for transfection should be highly pure, sterile, and free from contaminants such as endotoxin (lipopolysaccharides). Remove any traces of endotoxin using Mirus' MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal amount of DNA for transfection is usually within 1-3 µg per well of a 6-well plate. As a starting point, use 2 µg of DNA per well of a 6-well plate.

D. *Trans*IT®-HeLa Reagent to 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 to 4 µl per 1 µg DNA. The volume of reagent that gives the highest transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Tables 1 or 2 for recommended starting conditions.

E. MONSTER Reagent to DNA ratio - As a starting point, use 1-3 µl of MONSTER Reagent per 1 µg of DNA. The MONSTER Reagent can be titrated from 0.5 to 5 µl per 1 µg DNA, depending on the specific subtype. The ratio that provides the highest transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Tables 1 or 2 for recommended starting conditions.

F. Transfection incubation time - The optimal incubation time can be determined empirically by testing a range from 24-48 hours.

The protocols in Sections 2.2 and 2.3 are recommended for performing transfections with the *Trans*IT®-HeLaMONSTER® Transfection Kit in 6-well plates. When performing transfections in different sized wells, the amount of DNA, *Trans*IT®-HeLa Reagent, MONSTER Reagent, and culture medium should be scaled up or down in proportion to the surface area of the well.

2.2 Recommended Protocol for DNA Transfection in 6-well Plates

NOTE: Optimal transfection efficiencies can be achieved using the following protocol. An alternate protocol (Section 2.3) 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 with the lowest cellular toxicity.

Table 1. Starting conditions using the recommended protocol

Culture Vessel	24-well plate	12-well plate	6-well (35mm) plate
Surface Area	1.9 cm^2	3.8 cm^2	9.6 cm^2
Serum-free Media	50 µl	100 µl	200 µl
*Trans*IT®-HeLa Transfection Reagent	1-2 µl	2-4 µl	4-8 µl
DNA (1 µg/µl stock)	0.5 µl	1 µl	2 µl
MONSTER Reagent (as supplied) 1 µg/µl	0.25-0.75 µl	0.5-1.5 µl	1-3 µl
Complete Growth Media	500 µl	1000 µl	2500 µl

*All volumes in Table 1 are per well of the indicated size.

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10^5 cells in their complete growth medium per well of a 6-well plate) to obtain ~50-70% confluent the following day. [a]
2. Incubate the cells overnight.[b]

B. Complex Formation (perform this procedure immediately prior to transfection)

1. In a sterile plastic tube, add the *Trans*IT®-HeLa Reagent (2 to 4 µl per 1 µg DNA) directly into 200 µl of serum-free medium. Mix thoroughly by pipetting or vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1 to 3 µg per well) to the diluted *Trans*IT®-HeLa Reagent[c,e] and mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.
5. Add undiluted MONSTER Reagent (1 to 3 µl per 1 µg DNA) to the *Trans*IT®-HeLa Reagent/DNA complexes and mix by gentle pipetting.
6. Incubate at room temperature 5-20 minutes.
7. Add 2 ml (per well) of complete growth medium (prewarmed to 37°C) to the complexes in the tube.

C. Cell Preparation for Transfection in Complete Growth Medium

1. Remove the medium from the cells immediately prior to the addition of the complexes.
2. Add the complexes containing *Trans*IT®-HeLa Reagent/DNA/MONSTER Reagent/media mixture from step B to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 24-48 hours. [b]
4. Harvest cells and assay for gene expression.

   NOTE: The above incubation is designed for transfections performed with no media change. To perform a media change after a 4-24 hour incubation with the complexes, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours. [b,d]

2.3 Alternate Protocol for DNA Transfection in 6-well Plates

NOTE: This is an alternate protocol that uses a slightly modified procedure to apply the MONSTER Reagent directly to the cells. Choose the protocol that achieves the highest transfection efficiency with the lowest cellular toxicity.

Table 2. Starting conditions using the alternate protocol

Culture Vessel	24-well plate	12-well plate	6-well (35mm) plate
Surface Area	1.9 cm^2	3.8 cm^2	9.6 cm^2
Serum-free Media	50 µl	100 µl	200 µl
*Trans*IT®-HeLa Transfection Reagent	1-2 µl	2-4 µl	4-8 µl
DNA (1 µg/µl stock)	0.5 µl	1 µl	2 µl
MONSTER Reagent (diluted 10 fold) 0.1 µg/µl	2.5-7.5 µl	5-15 µl	10-30 µl
Complete Growth Media	500 µl	1000 µl	2500 µl

*All volumes in Table 1 are per well of the indicated size.

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10^5 cells in their complete growth medium per well of a 6-well plate) to obtain ~50-70% confluent the following day. [a]
2. Incubate the cells overnight.[b]

B. Complex Formation (perform this procedure immediately prior to transfection)

1. In a sterile plastic tube, add the *Trans*IT®-HeLa Reagent (2 to 4 µl per 1 µg DNA) directly into 200 µl of serum-free medium. Mix thoroughly by pipetting or vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1 to 3 µg per well) to the *Trans*IT®-HeLa Reagent[c,e] and mix by gentle pipetting.
3. Incubate at room temperature for 5-20 minutes.

C. Preparation of the MONSTER Reagent (perform this procedure just prior to complex formation or during complex incubation.)

1. In a sterile tube, dilute the required amount (see Table 1) of MONSTER Reagent 10-fold in sterile (DNase, RNase, pyrogen-free) water. (i.e. to transfect 1 µg of DNA, add 1 µl of MONSTER Reagent to 9 µl of sterile water.) Mix well.
   NOTE: Dilute only the required amount of MONSTER Reagent. DO NOT store diluted MONSTER Reagent. Immediately return the stock MONSTER Reagent to -20˚C after use.

D. Preparation for Transfection in Complete Growth Medium (perform this procedure just prior to complex formation or during complex incubation times.)

1. If necessary, remove the medium from the cells prepared in step A and replace with 2 ml per well of fresh complete growth medium.
2. Add the *Trans*IT®-HeLa Reagent/DNA complex mixture prepared in step B dropwise to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly. Do not swirl the plate.
3. Add the diluted MONSTER Reagent prepared in step C dropwise directly to the cells, immediately move the dish back and forth and from side to side to distribute the reagent evenly after each addition. Do not swirl the plate.
4. Incubate for 24-48 hours. [b]
   NOTE: The above incubation is designed for transfections performed with no media change. To perform a media change after a 4-24 hours incubation with the complexes, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.[b,d]
5. Harvest cells and assay for gene expression.

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[a] Since the optimal cell density (% confluence) for efficient transfection can vary between HeLa subtypes this should be determined for each subtype. Maintain the optimal seeding protocol between experiments for each cell type.

[b] Standard incubation conditions for mammalian cells are 37˚C in 5% CO_2.

[c] The *Trans*IT®-HeLa Reagent/DNA complex may form improperly if the complex formation medium contains serum, resulting in poor transfection efficiencies. Any serum free media can be used for complex formation, provided it does not contain polyanions such as dextran sulfate and heparin.

[d] The optimal incubation time should be determined empirically by testing a range of incubation times from 24-48 hours.

[e] For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 300-1000 µl.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-HeLa Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-HeLa Reagent to DNA ratio by titrating the reagent from 2 to 4 µl per 1 µg DNA. As a starting point, use 3 µl per 1 µg of DNA in 6-well plates. Use the amount that gives the best transfection efficiency and the lowest cellular toxicity for future transfections. Use 2 µg DNA per well of a 6-well plate.
- **Suboptimal amounts of MONSTER Reagent**
  Determine the optimal MONSTER Reagent to DNA ratio by titrating the stock reagent from 1 to 3 µl per 1 µg DNA. Choose the amount that gives the best transfection efficiency and the lowest cellular toxicity for future transfections.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, the transfection efficiencies may be adversely affected. Maintain a similar passage number between experiments to ensure reproducibility.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most HeLa cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for different subtypes in order to maximize transfection efficiency. Maintain the optimal density in future experiments for reproducibility.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media then add to cells in complete growth media (containing serum). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media and the media change is eliminated.
- **Poor quality of transfecting DNA**
  Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (lipopolysaccharides, LPS) using Mirus' MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT®-HeLa Reagent/DNA complex formation**
  Use serum-free medium when forming the complexes.
- **Inhibitor present during transfection**
  The presence of polyanions, such as dextran sulfate or heparin, can inhibit transfection. Use transfection medium that does not contain these polyanions. If polyanions are necessary, the transfection media can be replaced with the optimal cell growth media 24 hours post transfection.

High Cellular Toxicity

- **Excessive amounts of *Trans*IT®-HeLa Reagent/DNA complex mixture or MONSTER Reagent were used in transfection**
  Reduce the amount of appropriate reagent in the transfection. See Tables 1 or 2 for recommended starting conditions.
- **Media change or addition may be necessary**
  If incubating for 48-72 hours, it may be necessary to change the complete media 24 hours post-transfection. Alternatively, add additional complete media 4-24 hours post-transfection.
- **Cell density (% confluence) was not optimal at time of transfection**
  Allow cells to grow to a higher cell density and repeat the experiment.
- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (containing serum). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media and the media change is eliminated.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, they can be more sensitive to transfection reagents. Maintain a similar passage number between experiments to ensure reproducibility.
- **Complexes were not mixed thoroughly**
  Thoroughly mix complexes before they are added to the cells. If following Section 2.3, ensure the complexes are added in a dropwise fashion. Rock the dish back and forth and from side to side. Do not swirl or rotate the dish, as this may result in uneven distribution.

For specific questions or concerns, please contact Mirus Technical Support at 888.530.0801 techsupport@mirusbio.com
For a list of citations using Mirus products, please visit Technical Resources at (www.mirusbio.com).

4.0 RELATED PRODUCTS

For endotoxin removal from DNA:*

MiraCLEAN® Endotoxin Removal Kit (Product #5900)

For DNA tracking studies:

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

Additional transfection reagents:*

*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-293 Transfection Reagent (Product # MIR 2700)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Prostate Transfection Reagent (Product # MIR 2130)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo Gene Delivery Kits:*

*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)

RNA Interference Products:*

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
siXpress® PCR Vector Systems (Product # MIR 7300, 7301, 7302)
*Trans*IT-TKO® HTS-96 Plates (Product # MIR 2530, 2540, 2550, 2560, 2570)
Label IT® siRNA Tracker Intracellular Localization Kit (Product # MIR 7200,7201,7202,7203,7204,7205,7014,7015)

*These products are available in additional sizes.

The performance of this product is guaranteed for 1 year from the date of purchase if stored and handled properly.

Mirus Transfection Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811, 6,593,465 and patents pending.

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