*Trans*IT®-COS Transfection Kit

Product Name	Volume of *Trans*IT®-COS Reagent	Volume of COS Boss Reagent	Product No.
*Trans*IT®-COS Transfection Kit	0.4 ml	0.2 ml	MIR 2194
	1 ml	0.5 ml	MIR 2190
	5 ml (5 × 1 ml)	2.5 ml (5 × 0.5 ml)	MIR 2195
	10 ml (10 × 1 ml)	5 ml (10 × 0.5 ml)	MIR 2196

Each milliliter of *Trans*IT®-COS Transfection Kit (MIR 2190) 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®-COS Transfection Kit was developed by 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. COS cells (African green monkey kidney) are a prevalent cell line in the biological research field, therefore the specificity of the *Trans*IT®-COS 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. Transfections with the *Trans*IT® Reagents do not require media changes and can be carried out in serum-containing media. In addition, the *Trans*IT®-COS Transfection Kit is quality control tested on ATCC COS-7 cells. These significant features establish the *Trans*IT®-COS Transfection Kit as the product of choice for transfecting COS cells.

1.2 Specifications

Concentration: *Trans*IT®-COS Reagent: 2.22 mg/ml in 80% ethanol
COS Boss Reagent: 2 mg/ml in 80% ethanol

Storage: Store both reagents at -20˚C. Prior to use, warm both reagents 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 protocol described in Section 2.2 should result in efficient transfection of most COS cell subtypes; however, to ensure optimal results consider the following variables:

A. Media conditions - The *Trans*IT®- COS Transfection 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 COS cell subtypes at transfection is 50-70% confluence. Determine the optimal cell density for each COS 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 Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal amount of DNA for transfection is usually within 1 to 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®-COS Reagent to DNA ratio - As a starting point, use 3 µl of *Trans*IT®-COS Reagent per 1 µg of DNA. The optimal *Trans*IT®-COS Reagent to DNA ratio can be determined by titrating the reagent from 2 to 4 µl per 1 µg DNA. For future transfections use the volume of reagent that gives the highest transfection efficiency with the lowest cellular toxicity on similarly passaged cells. Refer to Table 1 for recommended starting conditions.

E. COS Boss Reagent to DNA ratio - As a starting point, use 1 µl of COS Boss Reagent per 1 µg of DNA. The COS Boss Reagent can be titrated from 0.5 to 1.5 µl per 1 µg DNA, depending on the specific subtype. For future transfections use the ratio that provides the highest transfection efficiency with the lowest cellular toxicity on similarly passaged cells. Refer to Table 1 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 protocol below is recommended for performing transfections with the *Trans*IT®-COS Transfection Kit in 6-well plates. When performing transfections in different sized plates, the amount of DNA, *Trans*IT®-COS Reagent, COS Boss Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish.

Table 1. Recommended starting conditions using the *Trans*IT®-COS Transfection Kit

Culture Vessel	24-well plate	12-well plate	6-well 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®-COS Transfection Reagent	1-2 µl	2-4 µl	4-8 µl
COS Boss Reagent 2 µg/µl	0.25-0.75 µl	0.5-1.5 µl	1-3 µl
DNA (1 µg/µl stock)	0.5 µl	1 µl	2 µl
Complete Growth Media	500 µl	1000 µl	2000 µl

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

2.2 Protocol for Transient Transfection in 6-well plates

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density (~1-3 x 10^5 cells in complete growth medium per ml) to obtain 50-70% confluence 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®-COS Reagent (2 to 4 per 1 µg DNA) directly into 200 µl of serum-free medium.[c,e] Mix thoroughly by pipetting or vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add COS Boss Reagent (0.5 to 1.5 µl per 1 µg DNA) to serum-free media from Step 1 and mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.
5. Add DNA (1 to 3 µg) to the diluted *Trans*IT®-COS Reagent/COS Boss Reagent mixture and mix by gentle pipetting.
6. Incubate at room temperature for 5-20 minutes.

   NOTE: To accommodate small pipetting volumes, the COS Boss Reagent can be diluted in 100% ethanol, immediately before use. Only dilute the necessary amount of COS Boss Reagent. DO NOT store diluted COS Boss Reagent.

C. Cell Preparation for Transfections in Complete Growth Medium

1. If necessary, remove the medium from the cells prepared in step A and replace it with 2 ml per well of fresh complete growth medium.
2. Add the *Trans*IT®-COS Reagent/COS Boss Reagent/DNA complex mixture prepared in step B dropwise to the cells. Gently rock the plate back and forth and from side to side to distribute the complexes evenly. Do not swirl the plate.
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]

---

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

b Standard incubation conditions for COS cells are 37˚C in 5% CO_2.

c The *Trans*IT®-COS Reagent/COS Boss Reagent/DNA complex may form improperly if the transfection 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 can be determined empirically by testing a range of incubation times from 4-48 hrs.

e For transfecting larger amounts of DNA, or if a precipitate forms upon adding the reagent, increase the volume of serum-free medium to 300-1,000 µl.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT®-COS Reagent to DNA ratio**
  Determine the optimal *Trans*IT®-COS 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 COS Boss Reagent**
  Determine the optimal COS Boss Reagent to DNA ratio by titrating the reagent from 0.5 to 1.5 µl per 1 µg DNA. Choose the amount that gives the best transfection efficiency and the lowest cellular toxicity for future transfections. As a starting point, use 1 µl of COS Boss Reagent per 1 µg of DNA.
- **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 COS 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 this 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) using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *Trans*IT®-COS Reagent/COS Boss 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 medium can be replaced with the optimal cell growth media 24 hours post transfection.

High Cellular Toxicity

- **Excessive amounts of *Trans*IT®-COS Reagent/DNA complex mixture or COS Boss Reagent were used in transfection**
  Reduce the amount of the appropriate reagent in the transfection. See Table 1 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. Ensure the complexes are added in a dropwise fashion. Rock the plate back and forth and from side to side. Do not swirl or rotate the plate, as this may result in uneven distribution.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801or techsupport@mirusbio.com

For a list of citations using Mirus Bio 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-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*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 with *Trans*IT-TKO® Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®- siQUEST™ Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

*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.

This product is sold to the Buyer with a limited license to use this product for research only. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio Corporation.

*Trans*IT, *Trans*IT-TKO, *Trans*IT-Neural, HeLaMONSTER, MiraCLEAN, HybQUEST, siXpress, and *Label* IT are registered trademarks of Mirus Bio Corporation.

Label IT Tracker and siQUEST are trademarks of Mirus Bio Corporation.