

***TransIT*[®]-CHO Transfection Kit**
 Product # MIR 2170, MIR 2174, MIR 2175, MIR 2176

Product Name	Volume of <i>TransIT</i> [®] -CHO Reagent	Volume of CHO Mojo Reagent	Product No.
<i>TransIT</i> [®] -CHO Transfection Kit	0.4 ml	0.28 ml	MIR 2174
	1 ml	0.70 ml	MIR 2170
	5 ml (5 × 1 ml)	3.5 ml (5 x 0.70 ml)	MIR 2175
	10 ml (10 × 1 ml)	7.0 ml (10 x 0.70 ml)	MIR 2176

1.0 INTENDED USE

The *TransIT*[®]-CHO Transfection Kit, which consists of a *TransIT*[®]-CHO Reagent and a CHO Mojo Reagent, was specifically developed to obtain maximal transfection efficiency and cell viability in CHO-K1 cells. MIR 2170 provides sufficient amounts of both reagents to perform up to 500 transfections in 35 mm wells.

2.0 DESCRIPTION

2.1 General Information

The *TransIT*[®]-CHO Transfection Kit was developed by the nucleic acid delivery specialists of Mirus Bio Corporation. This novel kit was specifically optimized to provide superior transfection efficiency in CHO-K1 cells without sacrificing cellular health. CHO-K1 (Chinese hamster ovary) cells have been moderately difficult to transfect, yet have remained a prevalent cell line in the biological research field. The specificity of the *TransIT*[®]-CHO Transfection Kit makes this product a desirable alternative to broad spectrum transfection reagents. The kit provides all the attributes of the trusted *TransIT*[®] Reagent line: high efficiency, low toxicity, simplicity of use, and reproducibility. Transfections with the *TransIT*[®] Reagents do not require media changes and can be carried out in serum-containing media. In addition, the *TransIT*[®]-CHO Transfection Kit is quality control tested on ATCC CHO-K1 cells. These significant features establish the *TransIT*[®]-CHO Transfection Kit as the product of choice for transfecting CHO-K1 cells.

2.2 Specifications

Concentration: *TransIT*[®]-CHO Reagent: 2.22 mg/ml in 80% ethanol
 CHO Mojo Reagent: 5 mg/ml in 100% ethanol

Storage: Store both reagents at -20°C. Prior to use, warm both the *TransIT*[®]-CHO Reagent and the CHO Mojo Reagent to room temperature and gently vortex to dissolve any precipitate that may have formed.

Stability: 6 months from the date of purchase when stored at -20°C

3. PROCEDURE

3.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each CHO-K1 subtype. The transfection protocols described in Section 3.2 should result in efficient transfection of most CHO-K1 cell subtypes; however, to ensure optimal results the following variables should be considered:

- A. Cell density (confluence) at transfection** - The recommended cell density for most CHO-K1 cell types at transfection is 60-90% confluence. The optimal cell density should be determined for each CHO-K1 cell subtype in order to maximize transfection efficiency. This density should be maintained in future experiments for reproducibility.

- B. DNA concentration for transfection** - DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. The optimal DNA concentration for transfection usually falls within the range of 1-3 μg per 35 mm dish. As a starting point, use 2 μg per 35 mm dish.
- C. *TransIT*[®]-CHO Reagent to DNA ratio** - As a starting point, use 2-3 μl of *TransIT*[®]-CHO Reagent per 1 μg of DNA. The optimal *TransIT*[®]-CHO Reagent to DNA ratio can be determined by titrating the reagent starting at 1-5 μl per μg DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Table 1 for recommended starting conditions.
- D. CHO Mojo Reagent to DNA ratio** - As a starting point, use 0.5 μl of CHO Mojo Reagent per 1 μg of DNA. The optimal CHO Mojo Reagent to DNA ratio can be determined by titrating the reagent from 0.25-2 μl per μg of DNA. The ratio that gives the best transfection efficiency with the lowest cellular toxicity should be used for future transfections on similarly passaged cells. Refer to Table 1 for recommended starting conditions.
- E. Transfection Incubation Time** - The optimal incubation time can be determined empirically by testing a range of incubation times from 4-48 hours.

The protocols below are recommended for performing transfections with the *TransIT*[®]-CHO Transfection Kit in 35 mm wells. When performing transfections in different sized dishes, the amounts of DNA, *TransIT*[®]-CHO Reagent, CHO Mojo Reagent, and culture medium should be scaled up or down in proportion to the surface area of the dish. For your convenience, we have supplied the following table of recommended starting conditions:

Table 1. Recommended starting conditions for using the *TransIT*[®]-CHO Transfection Kit:

Culture Vessel	6-well (35 mm) plate	12-well plate	24-well plate
Surface area	9.4 cm ²	3.8 cm ²	1.9 cm ²
<i>TransIT</i> [®] -CHO Reagent	4-8 μl	2-4 μl	1-2 μl
Complete Growth Media	2000 μl	1000 μl	500 μl
Opti-MEM [®] I or other serum-free media	200 μl	100 μl	50 μl
DNA (1 $\mu\text{g}/\mu\text{l}$ stock)	2 μl	1 μl	0.5 μl
CHO Mojo Reagent (5 $\mu\text{g}/\mu\text{l}$ stock)	1-3 μl	0.5-1.5 μl	0.25-0.75 μl

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

3.2 Protocol for Transient Transfection (in 35 mm wells)

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at an appropriate cell density ($\sim 2\text{-}4 \times 10^5$ cells in complete growth medium per 35 mm well) to obtain $\sim 60\text{-}90\%$ confluency 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 12 x 75 mm tube, add the *TransIT*[®]-CHO Reagent (1-5 μl per μg DNA) dropwise into 200 μl of serum-free medium^{c,e} (Opti-MEM[®] I or RPMI 1640 from Gibco BRL are recommended for mammalian cell types). Mix thoroughly by vortexing.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (1-3 μg per well) to the diluted *TransIT*[®]-CHO Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 5-20 minutes.
5. Add CHO Mojo Reagent (0.25-2 μl per μg of DNA) to the complex mixture and mix by gentle pipetting.
6. Incubate at room temperature for 5-20 minutes.

NOTE: To accommodate small pipetting volumes, the CHO Mojo Reagent can be diluted 5-fold to 1 mg/ml in 100% ethanol, immediately before use. Only dilute the required amount of CHO Mojo Reagent. DO NOT store diluted CHO Mojo Reagent.

C. Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *TransIT*[®]-CHO Transfection Kit yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated.

1. If necessary, remove the medium from the cells prepared in step A and replace it with 2 ml per well (see Table 1) of fresh complete growth medium.
2. Add the *TransIT*[®]-CHO Reagent/DNA/CHO Mojo Reagent 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.
3. 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 hour incubation with the complexes, replace the original medium with fresh complete growth medium, and incubate for an additional 24-48 hours.^{b,d}

4. Harvest cells and assay for reporter gene activity.

^a Since the optimal cell density (confluence) for efficient transfection can vary between CHO-K1 cell subtypes, maintain the same seeding protocol for subsequent experiments.

^b Standard incubation conditions for CHO-K1 cells are 37°C in 5% CO₂.

^c The *TransIT*[®]-CHO Reagent/DNA/CHO Mojo Reagent complex may form improperly if the transfection medium contains serum, resulting in poor transfection efficiencies.

^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 200-1,000 µl.

4.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *TransIT*[®]-CHO Reagent to DNA ratio**
Determine the optimal *TransIT*[®]-CHO Reagent to DNA ratio by titrating the reagent from 1- 5 µl per µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 2-3 µl of *TransIT*[®]-CHO Reagent per 1 µg of DNA.
- **Suboptimal amounts of CHO Mojo Reagent**
Determine the optimal CHO Mojo Reagent to DNA ratio by titrating the reagent from 0.25-2 µl per µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 0.5 µl of CHO Mojo Reagent per 1 µg of DNA.
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**
Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (LPS) using Mirus' MiraCLEAN™ Endotoxin Removal Kit (Product # MIR 5900).
- **Fetal calf serum present during *TransIT*[®]-CHO Reagent/DNA/CHO Mojo Reagent complex formation**
Use serum-free medium when forming the complexes. Transfections should be performed in the presence of serum.
- **Cell density (% confluence) not optimal at time of transfection**
The recommended cell density for most CHO-K1 cell types at the time of transfection is 60-90% confluence. However, it may be necessary to determine the optimal cell density for specialized experiments in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **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.
- **Reagent formed precipitate during storage**
The *TransIT*[®]-CHO Reagent may form a precipitate during long term storage at -20°C. Warm the reagent to room temperature and gently vortex to dissolve any precipitate.

High Cellular Toxicity

- **TransIT[®]-CHO Reagent/DNA/CHO Mojo Reagent complex mixture was not mixed thoroughly following addition to the cells**

Mix thoroughly to evenly distribute the complexes to all cells. Rocking the dish back and forth and from side to side is recommended. Do not swirl or rotate the dish, as this may result in uneven distribution.

- **Excessive amounts of TransIT[®]-CHO Reagent, DNA, or CHO Mojo Reagent were used in transfection**
Reduce the amount of appropriate reagent in the transfection.
- **Cell density was too low at time of transfection**
Grow cells to a higher cell density and repeat the experiment.

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

For a list of citations using Mirus' products, please visit the Technical Resources section of our website. (www.mirusbio.com)

5.0 BIBLIOGRAPHY

1. Budker, V. et al. (1996) Nature Biotechnology 14: 760-4.
2. Hagstrom, J. et al. (1996) Biochim. Biophys. Acta 1284: 47-55.
3. Fritz, J. D. et al. (1996) Human Gene Therapy 7: 1395-1404.

6.0 RELATED PRODUCTS

For determination of gene expression efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

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)

Additional transfection reagents:

TransIT[®]-LT1 and -LT2 Transfection Reagents (Product # MIR 2300, 2400)

TransIT[®]-Express Transfection Reagent (Product # MIR 2000)

TransIT[®]-HeLaMONSTER[™] Transfection Reagent (Product # MIR 2900)

TransIT[®]-293 Transfection Reagent (Product # MIR 2700)

TransIT[®]-Keratinocyte Transfection Reagent (Product # MIR 2800)

TransIT[®]-3T3 Transfection Reagent (Product # MIR 2180)

TransIT[®]-COS Transfection Reagent (Product # MIR 2190)

TransIT[®]-Insecta[™] Transfection Reagent (Product # MIR 2200)

TransIT[®]-TKO[®] siRNA Transfection Reagent (Product # MIR 2150)

TransIT[®]-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo Gene Delivery Kits:

TransIT[®]-In Vivo Gene Delivery System (Product # MIR 5100)

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

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

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