*Trans*IT-Neural® Transfection Reagent

Product # MIR 2140, MIR 2144, MIR 2145, MIR 2146

Product Name	Volume of *Trans*IT-Neural® Reagent	Product No.
*Trans*IT-Neural® Transfection Reagent	0.4 ml	MIR 2144
	1 ml	MIR 2140
	5 ml (5 × 1 ml)	MIR 2145
	10 ml (10 × 1 ml)	MIR 2146

MIR 2140 provides sufficient amounts to perform up to 500 transfections in 12-well plates. Note: the total number of transfections is dependent on the type of neural cell line transfected. Please refer to Tables 1 and 2 for starting recommendations.

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT-Neural® Transfection Reagent was developed by the nucleic acid delivery specialists of Mirus Bio Corporation. This novel formulation provides superior transfection efficiency in neural cells without sacrificing cell viability. Generally, neural cell types have been difficult to transfect, yet have remained important in neurobiology. The specificity of the *Trans*IT-Neural® Transfection Reagent makes this product a desirable alternative to broad spectrum transfection reagents. The reagent possesses 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-Neural® Transfection Reagent is quality control tested and performance assured using ATCC Neuro-2a cells. These significant features establish the *Trans*IT-Neural® Transfection Reagent as the product of choice for transfecting neural cells.

1.2 Neural Cell Lines Successfully Transfected by Mirus Bio Corporation

C6, Daoy, DBTRG-05MG, DI-TNC1, HCN-1A, Neuro-2a, PC-12, SK-N-MC, SVG p12

1.3 Product Specifications

Concentration: 1.25 mg/ml

Storage: Store at 4°C. Do not freeze.

Stability: 1 year from the date of purchase when stored at 4°C

2.0 PROCEDURE

2.1 Transfection Optimization

The key to successful transfection is careful optimization of reaction conditions for each neural cell type. The transfection protocols described in Section 2.2 should result in efficient transfection of most neural cell types. However, to ensure optimal results, the following variables should be considered:

A. Media conditions - *Trans*IT® Reagents yield improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) with no media change.

B. Cell density (% confluence) at transfection - The recommended cell density for most neural cell types at transfection is 50-70% confluence. The optimal cell density should be determined for each neural cell type in order to maximize transfection efficiency. This density should be maintained in future experiments for reproducibility.

C. DNA purity and concentration for transfection - DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin using Mirus Bio's MiraCLEAN™ Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection usually falls within the range of 0.5-2.0 µg per well of a 12-well plate. As a starting point, use 1 µg per well of a 12-well plate.

D. *Trans*IT-Neural® Reagent to DNA ratio - Refer to Tables 1 and 2 for recommended starting conditions on particular cell lines. The optimal *Trans*IT-Neural® Reagent to DNA ratio can be determined by titrating the reagent from 1-16 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.

D. Cell preparation protocols - The optimal cell preparation protocol can be determined by testing the different protocol options in Sections 2.2C1, C2 and C3. Choose the cell preparation protocol that gives the best transfection efficiency and the lowest cellular toxicity.

E. Transfection Incubation Time – Determine the optimal incubation time empirically by testing a range of incubation times from 4-48 hours..

The protocols below are recommended for performing transfections in 12-well plates. When performing transfections in different sized plates, the amount of DNA, *Trans*IT-Neural® Transfection Reagent, serum-free medium and culture medium should be scaled up or down in proportion to the surface area of the well.

Table 1. Recommended starting conditions using the *Trans*IT-Neural® Transfection Reagent on Neuro-2a cells:

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10 cm dish
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
Serum-free Media	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml
*Trans*IT-Neural® Reagent	0.09-0.28 µl	0.26-0.79 µl	0.5-1.5 µl	1-3 µl	2.5-7.5 µl	15-46 µl
DNA (1µg/µl stock)	0.1 µl	0.26µl	0.5 µl	1 µl	2.5 µl	15 µl
Complete Growth Media	0.092 ml	0.263 ml	0.500 ml	1.0 ml	2.5 ml	15.5 ml

*Surface areas are based on Greiner tissue culture plates and Falcon 10 cm dishes. All volumes in Table 1 are per one well of indicated size.

Table 2. Recommended starting conditions using *Trans*IT-Neural® Transfection Reagent on various neural cell lines:

Cell line	Tissue Source	Cell Classification	*Trans*IT-*Neural*® Reagent per 1 µg DNA
C6	rat, brain	glioma	8-12 µl
Daoy	human, brain, cerebellum	medulloblastoma	1-4 µl
DBTRG-05MG	human, brain	glioblastoma	2-4 µl
DI-TNC1	rat, brain, diencephalon	astrocyte	8-12 µl
HCN-1A	human, brain	cortical neuron	2-4 µl
Neuro-2a	mouse, brain	neuroblastoma	1-3 µl
PC-12	rat, adrenal gland	pheochromocytoma	1-2 µl
SK-N-MC	human, brain	neuroepithelioma	12-16 µl
SVG p12	human, brain	astroglia	1-2 µl

NOTE: Use 100 µl of serum-free media, 1 µg of DNA, and 1 ml of complete growth media per well of a 12-well plate with the recommended *Trans*IT-Neural® volumes indicated above in Table 2. The recommended levels of *Trans*IT-Neural® Reagent for the indicated cell lines are based on empirical testing performed at Mirus Bio. For different cell subtypes, the amount of reagent may need to be titrated to determine the level which achieves the highest transfection efficiency and cell viability.

2.2 Protocols for Transient Transfection (in 12-well plates)

A. Cell Plating

1. Approximately 24 hours prior to transfection, plate cells at a cell density (1-2 x 10^5 cells in complete growth medium per well of 12-well plate) 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-Neural® Reagent (1-16 µl per µg DNA; see Tables 1 and 2 for recommended starting conditions) directly into 100 µl of serum-free medium.[c,e] Mix thoroughly by gentle pipetting.
2. Incubate at room temperature for 5-20 minutes.
3. Add DNA (0.5-2.0 µg per well) to the diluted *Trans*IT-Neural® Reagent and mix by gentle pipetting.
4. Incubate at room temperature for 5-10 minutes.

C1. Standard Cell Preparation for Transfections in Complete Growth Medium

NOTE: The *Trans*IT®-Neural™ Transfection Reagent yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) and the media change is eliminated.

1. Add the *Trans*IT®-Neural™ Reagent/DNA complex mixture prepared in step B dropwise to the cells in media from step A above. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
2. Incubate for 4-48 hours. [b]
3. Harvest cells and assay as needed.

C2. Alternate Cell Preparation #1 for Transfections in Complete Growth Medium

NOTE: In some of the cell lines tested, including Neuro-2a and Daoy cells, the following alternate protocol improves transfection efficiency.

1. Gently remove the medium from the cells prepared in step A and replace it with 1 ml per well of a 12-well plate (see Table 1) of fresh complete growth medium.
2. Add the *Trans*IT®-Neural™ Reagent/DNA complex mixture prepared in step B dropwise to the cells containing fresh media. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
3. Incubate for 4-48 hours. [b]
4. Harvest cells and assay as needed.

C3. Alternate Cell Preparation #2 for Transfections in Complete Growth Medium

NOTE: In some of the cell lines tested, including SK-N-MC and DI-TNC1 cells, the following alternate protocol improves transfection efficiency.

1. Remove half of the volume (0.5 ml per well of a 12-well plate) of complete medium from each well of cells from step A.
2. Add 0.5 ml of fresh complete growth medium to the complex tube from step B (after the complexes have formed for 5-10 minutes). Mix thoroughly.
3. Add the *Trans*IT®-Neural™ Reagent/DNA/fresh complete media mixture to the cells. Gently rock the dish back and forth and from side to side to distribute the complexes evenly.
4. Incubate for 4-48 hours. [b]
5. Harvest cells and assay as needed.

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[a] Since the optimal cell density (confluence) for efficient transfection can vary between neural cell subtypes, maintain the same seeding protocol for subsequent experiments.

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

[c] The *Trans*IT-Neural® Reagent/DNA complex may form improperly if the complex formation 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.

3.0 TROUBLESHOOTING

Low Transfection Efficiency

- **Suboptimal *Trans*IT-Neural® Reagent to DNA ratio**
  Determine the optimal *Trans*IT-Neural® Reagent to DNA ratio by titrating the reagent from 1-16 µl per µg DNA. Choose the amount which gives the best transfection efficiency and the lowest cellular toxicity. As a starting point, use 2-8 µl of *Trans*IT-Neural® Reagent per 1 µg of DNA. Refer to Table 2 for starting recommendations in 12-well plates for specific cell lines.
- **Complexes were added to cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (serum-containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to the cells in complete growth media with no media change.
- **Cell density (% confluence) not optimal at time of transfection**
  The recommended cell density for most neural cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for different cell types in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.
- **Poor quality of transfecting DNA (DNA may be partially degraded or an inhibitor, such as an endotoxin, may be present in the preparation)**
  DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxin. Remove any traces of endotoxin (bacterial lipopolysaccharide) using the MiraCLEAN® Endotoxin Removal Kit (Product # MIR 5900). The optimal DNA concentration for transfection is 1-5 µg per well of a 6-well plate. As a starting point, use 2.5 µg per well of a 6-well plate.
- **Fetal calf serum present during *Trans*IT-Neural® Reagent/DNA complex formation**
  Use serum-free medium when forming the complexes. Transfections should be performed in the presence of serum.
- **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.
- **Cell morphology has changed**
  If the passage number of the cells is too high or too low, transfection efficiency can be adversely affected. Maintain a similar passage number between experiments to ensure reproducibility.

High Cellular Toxicity

- **Complexes were added to the cells in serum-free media**
  Form complexes in serum-free media, and add to cells in complete growth media (serum containing). Transfection efficiency is improved and cytotoxicity is decreased when the complexes are added to cells in complete growth media with no media change.
- ***Trans*IT-Neural® Reagent/DNA 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.
- **Cell density (% confluence) was not optimal at time of transfection**
  The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for each cell type in order to maximize transfection efficiency. Maintain this density in future experiments to ensure reproducibility.
- **Excessive amounts of *Trans*IT-Neural® Reagent or DNA were used in transfection**
  Reduce the amount of reagent or DNA in the transfection. See Tables 1 and 2 for recommended starting conditions.
- **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. We recommend maintaining a similar passage number between experiments to ensure reproducibility.

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 Bio products, please visit the Technical Resources section of our website 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 Plasmid 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®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*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®-293 Transfection Kit (Product # MIR 2700)
*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 Kit (Product # MIR 2130)
*Trans*IT®-mRNA Transfection Reagent (Product # MIR 2250)
*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)
*Trans*IT®-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
*Trans*IT®-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)
*Trans*IT®-QR Hydrodynamic Delivery Solution (Product # MIR 5240)
*Trans*IT®-QR Hydrodynamic Delivery Starter Kit (Product # MIR 5210)

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)
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.