

TransIT-Neural[®] Transfection Reagent

Protocol for MIR 2140, 2144, 2145, 2146



The Transfection Experts

INTRODUCTION

TransIT-Neural[®] Transfection Reagent is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in various neural cell types. Generally, neural cell types have been difficult to transfect, yet have remained important in neurobiology.

TransIT-Neural Reagent provides all the attributes of the trusted TransIT series of transfection reagents: high transfection efficiency, low toxicity, serum compatibility, simplicity of use and reproducibility. Transfection with TransIT-Neural Reagent does not require medium changes and can be carried out in serum-containing medium. TransIT-Neural is suitable for both transient and stable transfection.

SPECIFICATIONS

Storage	Store TransIT-Neural Reagent tightly capped at 4°C. Do not freeze. 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 TransIT-Neural to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

TransIT-Neural Transfection Reagent is supplied in **one** of the following formats.

Product No.	Quantity
MIR 2144	1 × 0.4 ml
MIR 2140	1 × 1.0 ml
MIR 2145	5 × 1.0 ml
MIR 2146	10 × 1.0 ml

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Purified plasmid DNA
- Serum-free medium (e.g. Opti-MEM[®] I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipets
- Reporter assay as required
- *Optional:* Selection antibiotic (e.g., G418 or Hygromycin B) for stable transfection

For Research Use Only.

BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each neural cell type to ensure successful transfections. The suggestions below yield high efficiency transfection using *TransIT-Neural* Transfection Reagent.

Table 1 presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density at transfection is 50–70% confluence. Determine the optimal cell density for each neural cell type 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.
- **Ratio of *TransIT-Neural* Reagent to DNA.** Determine the best *TransIT-Neural* Reagent:DNA ratio for each neural cell type. Vary the concentration of *TransIT-Neural* Reagent from 1–16 μ l per 1 μ g DNA to find the optimal ratio. Please refer to **Table 2** for recommended *TransIT-Neural* Reagent:DNA ratio for different neural cell types. Start with 3 μ l of *TransIT-Neural* Reagent per 1 μ g of DNA.
- **Complex formation conditions.** Prepare *TransIT-Neural* Reagent: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. The *TransIT-Neural* Reagent yields improved efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change. There is no need to perform a medium change to remove the transfection complexes.
- **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 serum and 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.



Do not use DNA prepared using miniprep kits for transfection.



Do not use serum or antibiotics in the medium during transfection complex formation.



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 *TransIT-Neural* need to be pipetted, dilute the reagent in 80% ethanol before each use to avoid pipetting errors. **Do not** store diluted *TransIT-Neural* Reagent.

Table 1. Recommended starting conditions for DNA transfections with *TransIT-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	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 μ l	263 μ l	0.5 ml	1.0 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 μ l	26 μ l	50 μ l	100 μ l	250 μ l	1.5 ml	1.9 ml
DNA (1 μ g/ μ l stock)	0.1 μ l	0.26 μ l	0.5 μ l	1 μ l	2.5 μ l	15 μ l	19 μ l
<i>TransIT-Neural</i> Reagent	0.3 μ l	0.78 μ l	1.5 μ l	3 μ l	7.5 μ l	45 μ l	57 μ l

Table 2. Recommended starting conditions for DNA transfections with *TransIT-Neural* Transfection Reagent on various neural cell types.

Cell type	Description	<i>TransIT-Neural</i> Reagent per 1 µg DNA
C6	Rat glioma cell line	8–12 µl
Daoy	Human medulloblastoma cell line	1–4 µl
DBTRG-05MG	Human glioblastoma cell line	2–4 µl
DI-TNC1	Rat brain astrocytes	8–12 µl
HCN-1A	Human cortical neurons	2–4 µl
Neuro-2a	Mouse neuroblastoma cell line	1–3 µl
PC-12	Rat pheochromocytoma cell line	1–2 µl
SK-N-MC	Human neuroepithelioma cell line	12–16 µl
SVG p12	Human brain astroglia	1–2 µl

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections using *TransIT-Neural* Transfection Reagent 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, *TransIT-Neural* Reagent, DNA and complete culture medium based on the surface area of the cell culture vessel (see **Table 1** on Page 2).

Transient plasmid 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 50–70% confluent prior to transfection.

For adherent cells: Plate cells at a density of $2-6 \times 10^5$ cells/well.

For suspension cells: Plate cells at a density of $8-10 \times 10^5$ cells/well.

2. Incubate cell cultures overnight.

B. Prepare *TransIT-Neural* Reagent:DNA complex (Immediately before transfection)

1. Warm *TransIT-Neural* 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 2.5µg (2.5 µl of a 1 µg/µl stock) plasmid DNA. Pipet gently to mix completely.
4. Add 7.5µl *TransIT-Neural* Reagent to the diluted DNA mixture. For further optimization of your cell type, test additional levels of the *TransIT-Neural* Reagent (please refer to and Table 2 on Page 3). Pipet gently to mix completely.
5. Incubate at room temperature for 15–30 minutes to allow sufficient time for complexes to form.



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



Warm *TransIT-Neural* to room temperature and vortex gently before each use.

C. Distribute the complexes to cells in complete growth medium

1. Add the *TransIT-Neural* Reagent:DNA complexes 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 *TransIT-Neural* Reagent: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.

D. Alternate protocol #1 for transfections in complete growth medium

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 2.5 ml per well of a 6-well plate (please refer to Table 1 on Page 2) of fresh complete growth medium.
2. Add the *TransIT-Neural* Reagent:DNA complexes (prepared in Step B) 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 *TransIT-Neural* Reagent:DNA complexes.
4. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
5. Harvest cells and assay as required.

E. Alternate protocol #2 for transfections in complete growth medium

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 (1.25 ml per well of a 6-well plate) of complete medium from each well of cells from step A.
2. Add 1.25 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 *TransIT-Neural* Reagent:DNA: fresh complete medium mixture drop-wise to different areas of the wells.
4. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *TransIT-Neural* Reagent:DNA complexes.
5. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.
6. Harvest cells and assay as required.



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

For generating stable cell transfectants, passage the cells 24–48 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 for selection of cells that have undergone stable integration of DNA.

TROUBLESHOOTING GUIDE

Problem	Solution
LOW PLASMID DNA TRANSFECTION EFFICIENCY	
TransIT-Neural Reagent was not mixed properly	Warm <i>TransIT-Neural</i> to room temperature and vortex gently before each use.
Suboptimal <i>TransIT-Neural</i> Reagent:DNA ratio	Determine the best <i>TransIT-Neural</i> Reagent:DNA ratio for each cell type. Titrate the <i>TransIT-Neural</i> Reagent from 1–16 μ l per 1 μ g 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 μ g/well of a 6-well plate. Start with 2.5 μ g/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of <i>TransIT-Neural</i> Transfection Reagent 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 <i>TransIT-Neural</i> Reagent:DNA complexes in serum-free growth medium. We recommend Opti-MEMI 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 according to Table 1 on page 2 including serum-free medium, <i>TransIT-Neural</i> Reagent and plasmid DNA. 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.
Proper experimental controls were not included	To verify efficient transfection, use <i>TransIT-Neural</i> Reagent 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’ <i>Label IT[®] Tracker[™]</i> Intracellular Nucleic Acid Localization Kit to label the target plasmid or Mirus’ pre-labeled <i>Label IT</i> Plasmid Delivery Controls (please refer to Related Products on Page 7)

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add <i>TransIT-Neural</i> Reagent:DNA complexes drop-wise to different areas of the wells containing the 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 <i>TransIT-Neural</i> Reagent:DNA 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 <i>TransIT-Neural</i> :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 neural cell type to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most neural cell types, 50–70% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on the 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
- TransIT[®]-2020 Transfection Reagent
- TransIT-PRO[™] Transfection Kit
- TransIT[®] Cell Line Specific Transfection Reagents and Kits
- TransIT[®]-In Vivo Gene Delivery Kits
- TransIT[®]-LT1 Transfection Reagent
- TransIT[®]-QR and TransIT[®]-EE Delivery Solutions and Kits

For details on the above mentioned products, visit www.mirusbio.com or www.TheTransfectionExperts.com.

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