*Trans*IT®-BrCa Transfection Reagent

Protocol for MIR 5500, 5504, 5505, 5506

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/5500

INTRODUCTION

*Trans*IT®-BrCa Transfection Reagent is specifically optimized to provide exceptional transfection efficiency of plasmid DNA in breast cancer and related cell types including: MCF-7, MDA-MB-231 and T47D cells. *Trans*IT-BrCa provides all the attributes of the trusted *Trans*IT series of transfection reagents: high transfection efficiency, low toxicity, serum compatibility, and simplicity of use. Transfections with *Trans*IT-BrCa Reagent do not require medium changes and can be carried out in serum-containing medium. *Trans*IT-BrCa is suitable for both transient and stable transfection and can be used for multiple applications such as gene expression, shRNA expression and promoter analyses.

SPECIFICATIONS

Storage	Store *Trans*IT-BrCa Reagent tightly capped at -20°C. ***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 *Trans*IT-BrCa to room temperature and vortex gently before each use.

MATERIALS

Materials Supplied

*Trans*IT-BrCa Transfection Reagent is supplied in ***one*** of the following formats.

Product No.	Quantity
MIR 5504	1 × 0.4 ml
MIR 5500	1 × 1.0 ml
MIR 5505	5 × 1.0 ml
MIR 5506	10 × 1.0 ml

Materials required, but not supplied

- Cultured breast cancer or related 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 breast cancer cell subtype to ensure successful transfections. The suggestions below yield high efficiency transfection in MCF-7, MDA-MB-231 and T47D cells using *Trans*IT-BrCa Transfection Reagent. **Table 1** presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection.** The recommended cell density for most breast cancer cell subtypes is 60–80% confluence at the time of transfection. Due to the clumpy nature of MCF-7 cells, there is a non-linear relationship between cell number and cell density causing the cell confluence to appear lower. The recommended cell density for MCF-7 cells is 40-60%. Divide cells 18–24 hours before transfection to ensure that the cells are actively dividing 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 might 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 *Trans*IT-BrCa Reagent to DNA.** Determine the best *Trans*IT-BrCa Reagent:DNA ratio for each cell type. Start with 2 µl of *Trans*IT-BrCa Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-BrCa Reagent from 1.5–4 µl per 1 µg DNA to find the optimal ratio. **Table 1** provides recommended starting conditions based on cell culture vessel size.
- **Complex formation conditions.** Prepare *Trans*IT-BrCa 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 *Trans*IT-BrCa 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 used, 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.

Table 1. Recommended starting conditions for DNA transfections with *Trans*IT-BrCa Transfection Reagent.

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 µl	263 µl	0.5 ml	1.0 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
DNA (1 µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
*Trans*IT-BrCa Reagent	0.2 µl	0.52 µl	1 µl	2 µl	5 µl	30 µl	38 µl

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 *Trans*IT-BrCa need to be pipetted, dilute the reagent in serum-free medium before each use to avoid pipetting errors. ***Do not*** store diluted *Trans*IT-BrCa Reagent.

PLASMID DNA TRANSFECTION PROTOCOL

The following procedure describes how to perform plasmid DNA transfections using *Trans*IT-BrCa 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, *Trans*IT-BrCa 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 2.5-5.0 × 10^5 cells in 2.5 ml complete growth medium per well in a 6-well plate. Ideally cells should be 60–80% confluent prior to transfection.
   ***For MCF-7 cells*:** The recommended cell density is 40-60%. (Please refer to "Before You Start on Page 2.)
2. Incubate cell cultures overnight.

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

B. Prepare *Trans*IT-BrCa Reagent:DNA complex (Immediately before transfection)

1. Warm *Trans*IT-BrCa 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 2.5 µg (2.5 µl of a 1 µg/µl stock) plasmid DNA.
4. Pipet gently to mix completely.
5. Add 5 µl *Trans*IT-BrCa Reagent to the diluted DNA mixture.
6. Pipet gently to mix completely.
7. Incubate at room temperature for 15–30 minutes.

Warm *Trans*IT-BrCa to room temperature and vortex gently before each use.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-BrCa Reagent:DNA complexes (prepared in Step B) 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 *Trans*IT-BrCa 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.

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

For generating stable cell transtectants, passage the cells 48-72 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	
*Trans*IT-BrCa Reagent was not mixed properly	Warm *Trans*IT-BrCa to room temperature and vortex gently before each use.
Suboptimal *Trans*IT-BrCa Reagent:DNA ratio	Determine the best *Trans*IT-BrCa Reagent:DNA ratio for each cell type. Titrate the *Trans*IT-BrCa Reagent from 1.5-4 µl per 1 µg DNA. Refer to "Before You Start" on Page 2.
Suboptimal DNA concentration	Determine the DNA concentration accurately. 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 µg/well of a 6-well plate. Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of *Trans*IT-BrCa Transfection Reagent accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. We recommend using Mirus 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 might contain high levels of endotoxin.
Inhibitor present during transfection	Serum and antibiotics inhibit transfection complex formation. Prepare *Trans*IT-BrCa Reagent:DNA complexes in serum-free growth medium. We recommend Opti-MEM I 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.24–72 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 media, *Trans*IT-BrCa and plasmid DNA. Precipitation maybe 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 *Trans*IT-BrCa 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 *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid or prelabeled *Label* IT Plasmid Delivery Controls (please refer to Related Products on Page 10)

TROUBLESHOOTING GUIDE continued

Problem	Solution
HIGH CELLULAR TOXICITY	
Transfection complexes and cells not mixed thoroughly after complex addition	Add *Trans*IT-BrCa 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 *Trans*IT-BrCa 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 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 might 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 *Trans*IT-BrCa:DNA ratio by using carrier DNA such as an empty non-coding cloning vector.
Cell density not optimal at time of transfection	Determine the best cell density for each breast cancer cell type to maximize transfection efficiency. Use this cell density in subsequent experiments to ensure reproducibility. Typically 60–80% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability and/or transfection efficiencies depending on the subtype.
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
- Cell Culture Antibiotic Solutions
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- TransIT-siQUEST® Transfection Reagent
- TransIT-TKO® Transfection Reagent

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

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.mirusbio.com/ra

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