

NIMT[®]FeOfection | PURPLE

Product Description

Product no R0-FS1-010, R0-FS1-050, R0-FS1-250.
Transfection agent.

Storage

NIMT[®]FeOfection | PURPLE is shipped on ice and it should be stored at 2-8° C upon arrival.
NIMT[®]FeOfection | PURPLE is for R&D use only.

Quality Control

NIMT[®]FeOfection | PURPLE is tested to ensure lot-to-lot consistency. Functionality of NIMT[®]FeOfection | PURPLE is tested by transfection of siRNA targeted to a common non-essential housekeeping gene such as glyceraldehydes-3-phosphate dehydrogenase (GAPDH) or lamin A/C and appropriate controls into a cell line.

NIMT[®]FeOfection | PURPLE is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycolate medium.

Introduction

NIMT[®]FeOfection | PURPLE is specially designed for efficient siRNA delivery into mammalian cells. NIMT[®]FeOfection | PURPLE is a transfection agent based on lipid coated nanoparticles with an iron oxide core formulated to yield high transfection efficiency while minimizing cell cytotoxicity. The nanoparticles are constructed from solid iron oxide cores that are coated with specific cationic lipid formulations to facilitate siRNA binding and cellular uptake.

General Guidelines

Cell Culture

For commercially available cell lines we recommend following the suppliers guidelines regarding culture medium and supplement as well as subculturing and seeding conditions.

Cell conditions before transfection:

- ✓ Cells should be in their logarithmic growth phase.
- ✓ Antibiotics can be included in the medium used during transfection and the subsequent incubation.
- ✓ Serum can be included in the medium used during transfection and the subsequent incubation.

Transfection

The following parameters need to be optimized for a successful knockdown with low toxicity

- ✓ Plating density
- ✓ NIMT[®]FeOfection | PURPLE concentration
- ✓ siRNA concentration
- ✓ Time of transfection

All procedures should be performed in a laminar flow hood using sterile technique. It is important to include a number of adequate controls i.e negative control siRNA (non-targeting siRNA, positive control siRNA (siRNA targeting a housekeeping or reporter gene), Mock (no siRNA) and untreated cells. All transfections should be performed in triplicate.

siRNA

Use 0.6-6 pmol siRNA per well in a 96-well format to start out with. Generally a higher concentration of siRNA will give a higher knockdown but the off-target effects will also increase.

Serum

NOTE! Do not use serum free media during the complex formation, this may have a negative effect on the cells proliferation. The best transfection efficiency is reached if double distilled water is used.

Transfection Protocol

Transfection protocol for NIMT[®]FeOfection | PURPLE and siRNA - ADHERENT CELLS

We strongly recommend optimizing the NIMT[®]FeOfection | PURPLE and siRNA concentrations used for achieving optimal knockdown with minimal toxicity. Cells can be either forward or reverse transfected depending upon cell line and application. Reverse transfection is faster and will save one day of experiment time. The optimization protocols below are guidelines for transfection in a 96-well plate.

Forward Transfection Optimization Protocol

The amount of NIMT[®]FeOfection|PURPLE used per well is based on a siRNA content of 0.6-6 pmol per well in a 96-well plate.

1. One day before transfection plate cells in 100 µl medium in a 96-well plate so that they are 20-50% confluent* on the day of transfection.
2. Dilute NIMT[®]FeOfection|PURPLE ten times in RNase free double distilled (dd) H₂O.
3. Add the diluted NIMT[®]FeOfection|PURPLE to RNase free ddH₂O to get the desired concentration according to table 1.
4. Dilute siRNA in a separate tube to the desired concentration according to table 1.
5. Add diluted siRNA to NIMT[®]FeOfection|PURPLE. Mix contents by pipetting carefully up and down and incubate for 10-20 min at room temperature.
6. Add 20 µl of NIMT[®]FeOfection|PURPLE /siRNA complexes to cells.
7. Incubate cells at 37°C in 5% CO₂ for 24-48 hrs (mRNA analysis) or 48-96 hrs (protein analysis) prior to evaluation of gene knockdown.

Table 1. Optimization of transfection

Sample	NIMT [®] FeOfection (diluted 10 times) (µl)	RNase Free H ₂ O (µl)	siRNA (60-600 nM) (µl)	Final volume (µl)	End siRNA Concentration (nM)
1	1.5	8.5	10	20	5-50
2	3	7	10	20	5-50
3	5	5	10	20	5-50
4	7	3	10	20	5-50
5 (Mock)	7	13	-	20	5-50

*Optimal cell densities will vary with different cell types and will need to be determined empirically.

Reverse Transfection Optimization Protocol

The amount of NIMT[®]FeOfection|PURPLE used per well is based on a siRNA content of 0.6-6 pmol per well in a 96-well plate.

1. Dilute NIMT[®]FeOfection|PURPLE ten times in RNase free double distilled (dd) H₂O.
2. Add the diluted NIMT[®]FeOfection|PURPLE to RNase free ddH₂O to get the desired concentration according to table 1.
3. Dilute siRNA in a separate tube to the desired concentration according to table 1.
4. Add diluted siRNA to NIMT[®]FeOfection|PURPLE. Mix contents by pipetting carefully up and down and incubate for 10-20 min at room temperature.
5. Add 20 µl of NIMT[®]FeOfection|PURPLE /siRNA complexes to wells in a 96-well plate.
6. Dilute cells in growth medium so that 100 µl contains the appropriate number of cells to give 30-70% confluence 24 hrs after transfection.
7. To each well with NIMT[®]FeOfection|PURPLE and siRNA complexes add 100 µl of diluted cells.
8. Incubate cells at 37°C in 5% CO₂ for 24-48 hrs (mRNA analysis) or 48-96 hrs (protein analysis) prior to evaluation of gene knockdown.

Transfection protocol for NIMT[®]FeOfection|PURPLE and siRNA - SUSPENSION CELLS

We strongly recommend optimizing the NIMT[®]FeOfection|PURPLE and siRNA concentrations used for achieving optimal knockdown with minimal toxicity. The optimization protocols below are guidelines for transfection in a 96-well plate. The amount of NIMT[®]FeOfection|PURPLE used per well is based on a siRNA content of 0.6-6 pmol per well in a 96-well plate.

1. On the day of transfection seed 4-8×10⁴ cells in a 96-well plate in 100 µl growth media.
2. Dilute NIMT[®]FeOfection|PURPLE ten times in RNase free double distilled (dd) H₂O.
3. Add the diluted NIMT[®]FeOfection|PURPLE to RNase free ddH₂O to get the desired concentration according to table 1.
4. Dilute siRNA in a separate tube to the desired concentration according to table 1.
5. Add diluted siRNA to NIMT[®]FeOfection|PURPLE. Mix contents by pipetting carefully up and down and incubate for 10-20 min at room temperature.
6. Add 20 µl of NIMT[®]FeOfection|PURPLE /siRNA complexes to cells.
7. Incubate cells at 37°C in 5% CO₂ for 24-48 hrs (mRNA analysis) or 48-96 hrs (protein analysis) prior to evaluation of gene knockdown.

Scaling Up

To transfect cells in other formats, vary the amounts of NIMT[®]FeOfection|PURPLE, siRNA and media according to table 2. The volumes and amounts given are only recommendations and should be optimized for optimal transfection efficiencies.

Table 2: Recommended volumes and amounts of transfection reagents for different cell culture formats.

Culture Formate	Plating volume (µl)	NIMT [®] FeOfection (diluted 10 times) (µl)	RNase Free H ₂ O (µl)	siRNA µl (pmol)	Final volume added per well (µl)	End siRNA concentration in well
96-well	100	1.5-7	8.5-3	10 (0.6-6)	20	5-50
48-well	200	3-14	17-6	20 (1.2-12)	40	5-50
24-well	500	7.5-35	42.5-15	50 (3-30)	100	5-50
12-well	1000	15-70	85-30	100 (6-60)	200	5-50
6-well	2500	37.5-175	212.5-75	250 (15-150)	500	5-50

Related Genovis Products

NIMT [®] FeOfection YELLOW	T0-FF1-050
NIMT [®] FeOfection PINK	T0-FF2-050
NIMT [®] FeOfection TESTKIT	T1-FFS-003

References

Freshney RI, Culture of Animal Cells A Manual of Basic Technique, 5th Ed., Wiley-Liss.

Elbashir SM, et al (2001) *Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells* Nature 411: 494-498.

Harborth, J, et al. (2001) *Identification of essential genes in cultured mammalian cells using small interfering RNAs* J Cell Sci 114(249: 4557-4565.

Product References

Application notes

NIMT[®]FeOfection in siRNA delivery into HEK293 cells.

Efficient Delivery of siRNA in Human Renal Cell Carcinoma Cell Line Using NIMT[®] FeOfection.

Knockdown of BACE-1, an Alzheimer's Disease Drug Target, with NIMT[®] FeOfection.