



rnaexact@rnaexact.com

Tel + 372 5626 0622

## MIRFECT (Cat # 001)

### TRANSFECTION PROTOCOL

#### Description

MIRFECT is a transfection reagent, which is specifically developed for the delivery of short oligonucleotides, such as miRNA mimics and siRNAs. MIRFECT forms nanocomplexes with unmodified and modified miRNA mimics and siRNAs and transfects nearly 100% of the cells. MIRFECT is efficient in the transfection of wide variety of cell-lines and primary cells.

#### Preparation of MIRFECT stock solution

Prepare MIRFECT transfection reagent stock solution as described below:

1. Reconstitute one vial of MIRFECT in 1 ml sterile ddH<sub>2</sub>O provided.
2. Mix and vortex the vial with reconstituted MIRFECT to ensure that the reagent is entirely dissolved.
3. Aliquot and store stock solutions at +4 °C. Stock solutions are stable at this condition at least 6 months. For longer storage, freeze at -20 °C, but avoid multiple freeze-thaw cycles. Keep at +4 °C after thawing.

#### Transfection procedure

These instructions are for transfecting cells in 12-well tissue culture plates with surface area of 4 cm<sup>2</sup>. For transfections in tissue culture plates with a surface area other than 4 cm<sup>2</sup>, please adapt amounts according to the Table 1.

Day 0:

Seed cells into a 12-well tissue culture plate at 50-70% confluency in 1000 µl normal growth medium per well.

Day 1:

1. Mix 30 pmol miRNA mimic/siRNA in volume of 100 µl OptiMEM, vortex briefly.
2. Add 2 µl of MIRFECT, vortex briefly.
3. To form MIRFECT-miRNA/siRNA nanocomplexes, incubate 30 min at room temperature.
4. Add 900 µl of normal growth media to the nanocomplexes and vortex briefly.
5. Remove old normal growth media from the cells.
6. Add new growth media containing MIRFECT-miRNA/siRNA transfection complexes to the cells.
7. Incubate for the following applications for 24-72 hours.

**Table 1. Scaling**

Plate	miRNA/siRNA	MIRFECT ( $\mu$ l)	OptiMEM ( $\mu$ l)	Normal growth media (ml)
24-well	15 pmol	1	50	0.4
12-well	30 pmol	2	100	0.9
6-well	60 pmol	4	200	1.8
$\varnothing$ 10 cm plate	150 pmol	10	1000	9.0

**Materials required but not provided**

Opti-MEM® I Reduced Serum Medium (Cat # 31985062, Thermo Fisher Scientific)

**Additional comments**

- As the transfection with MIRFECT is relatively fast, the growth media containing MIRFECT-miRNA/siRNA transfection complexes can be removed and replaced with normal growth media after 6 hour or next day.
- 50-70% confluency corresponds to  $5 \times 10^4$  human primary keratinocytes per one well
- Similar to other transfection reagents, MIRFECT slightly inhibits cell proliferation.
- MIRFECT is less toxic when compared with lipofection. Toxicity is enhanced at low cell concentration.
- MIRFECT is efficient in the transfection of common cell-lines, including HeLa, HEK293, PPC-1, HaCaT, A549, BEAS-2B, CCD19Lu and THP-1.
- MIRFECT is efficient in the transfection of wide variety of primary cells, such as human primary keratinocytes, primary skin fibroblasts, melanocytes, monocytes, macrophages and dendritic cells.