

## mmu-miR-654-5p inhibitor

Cat. No.:	HY-RI03442
Molecular Weight:	6568.35
Target:	MicroRNA
Pathway:	Epigenetics
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.

### BIOLOGICAL ACTIVITY

#### Description

mmu-miR-654-5p inhibitors are chemically-modified oligonucleotides that hybridize with mature miRNAs. The miRNA inhibitors have full-length nucleotide 2'-methoxy modification. The miRNA inhibitors strongly compete with mature miRNAs to prevent the complementary pairing of miRNAs and their target genes, thereby inhibiting miRNAs from functioning.

#### In Vitro

##### 1. miRNA Resuspension

1.1 Briefly centrifuge the tube to ensure that the dried miRNA is at the bottom of the tube.

1.2 Resuspend the miRNA using nuclease free water to generate 20  $\mu$ M stock solution.

For 5 nmol miRNA: add 250  $\mu$ L nuclease free water.

For 20 nmol miRNA: add 1000  $\mu$ L nuclease free water.

1.3 Aliquot miRNAs into one or more tubes to limit the number of freeze-thaw cycles (<5).

1.4 Store at or below -20°C or -80°C in a non-frost-free freezer until use.

##### 2. Prepare cells

2.1 Inoculate cells in advance for cell transfection.

Note: The viability and general health of cells prior to transfection significantly affect transfection result.

##### 3. Transfection

3.1 Prepare transfection mix A and B.

For per well of a 6-well plate: A: 120  $\mu$ L serum-free medium + 5  $\mu$ L miRNA inhibitors; B: 121  $\mu$ L serum-free medium + 4  $\mu$ L PolyFast Transfection Reagent (HY-K1014).

For per well of a 24-well plate: A: 23.75  $\mu$ L serum-free medium + 1.25  $\mu$ L miRNA inhibitors; B: 24  $\mu$ L serum-free medium + 1  $\mu$ L PolyFast Transfection Reagent (HY-K1014).

For per well of a 96-well plate: A: 4.75  $\mu$ L serum-free medium + 0.25  $\mu$ L miRNA inhibitors; B: 4.8  $\mu$ L serum-free medium + 0.2  $\mu$ L PolyFast Transfection Reagent (HY-K1014).

Note: The recommended working concentration is 100nM for miRNA inhibitors. miRNA function can vary greatly, depending on the miRNA, the cell line, and the chosen analysis method. To determine the concentration that provides optimal results, optimization experiments using varying mimic/inhibitor concentrations should be performed. The optimized range suggests changing the miRNA concentration in the range of 20 to 500nM.

If other transfection reagents are used, the amount of transfection reagent needs to be adjusted according to the specific situation.

3.2 Mix the diluted PolyFast Transfection Reagent and miRNA gently. Incubate at room temperature for 15 minutes.

3.3 Remove culture medium from cells, wash with PBS.

3.4 Add transfection mix (A+B) to cells.

For per well of a 6-well plate: add 1750  $\mu$ L fresh medium without Pen/Strep, then add 250  $\mu$ L of the transfection mix (A+B) to the well, and mix well.

For per well of a 24-well plate: add 450  $\mu$ L fresh medium without Pen/Strep, then add 50  $\mu$ L of the transfection mix (A+B) to the well, and mix well.

For per well of a 96-well plate: add 90  $\mu$ L fresh medium without Pen/Strep, then add 10  $\mu$ L of the transfection mix (A+B) to the well, and mix well.

3.5 Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells. The medium can be replaced with fresh serum-containing medium after 6 hours if necessary.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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