hsa-miR-4443 mimic

Cat. No.:	HY-R01047
Molecular Weight:	10766.52
Target:	MicroRNA
Pathway:	Epigenetics
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.

BIOLOGICAL ACTIVITY

Description	hsa-miR-4443 mimics are small, chemically synthesized double-stranded RNAs that mimic endogenous miRNAs and enable miRNA functional analysis by up-regulation of miRNA activity.
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 μM stock solution. For 5 nmol miRNA: add 250 μL nuclease free water. For 20 nmol miRNA: add 1000 μL 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. 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 μL serum-free medium + 5 μL miRNA mimics; B: 121 μL serum-free medium + 4 μL PolyFast Transfection Reagent (HY-K1014). For per well of a 24-well plate: A: 23.75 μL serum-free medium + 1.25 μL miRNA mimics; B: 24 μL serum-free medium + 1 μL PolyFast Transfection Reagent (HY-K1014). For per well of a 96-well plate: A: 4.75 μL serum-free medium + 0.25 μL miRNA mimics; B: 4.8 μL serum-free medium + 0.2 μL PolyFast Transfection Reagent (HY-K1014). Note: The recommended working concentration is 50nM for miRNA mimics. 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 10 to 200nM.

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

3.2 Mix A and B 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 µL fresh medium without Pen/Strep, then add 250 µL of the transfection mix (A+B) to the well, and mix well.

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

For per well of a 96-well plate: add 90 µL fresh medium without Pen/Strep, then add 10 µL 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.

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