

## mmu-miR-499-3p mimic

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|--------------------------|---|
| <b>Cat. No.:</b>         | HY-R03231   |
| <b>Molecular Weight:</b> | 14592.8   |
| <b>Target:</b>           | MicroRNA  |
| <b>Pathway:</b>          | Epigenetics   |
| <b>Storage:</b>          | Please store the product under the recommended conditions in the Certificate of Analysis. |

### BIOLOGICAL ACTIVITY

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| <b>Description</b> | mmu-miR-499-3p mimics are small, chemically synthesized double-stranded RNAs that mimic endogenous miRNAs and enable miRNA functional analysis by up-regulation of miRNA activity.  |
| <b>In Vitro</b>    | <ol style="list-style-type: none"> <li>1. miRNA Resuspension             <ol style="list-style-type: none"> <li>1.1 Briefly centrifuge the tube to ensure that the dried miRNA is at the bottom of the tube.</li> <li>1.2 Resuspend the miRNA using nuclease free water to generate 20 <math>\mu</math>M stock solution.                 <p>For 5 nmol miRNA: add 250 <math>\mu</math>L nuclease free water.</p> <p>For 20 nmol miRNA: add 1000 <math>\mu</math>L nuclease free water.</p> </li> <li>1.3 Aliquot miRNAs into one or more tubes to limit the number of freeze-thaw cycles (&lt;5).</li> <li>1.4 Store at or below -20°C or -80°C in a non-frost-free freezer until use.</li> </ol> </li> <li>2. Prepare cells             <ol style="list-style-type: none"> <li>2.1 Inoculate cells in advance for cell transfection. The viability and general health of cells prior to transfection significantly affect transfection result.</li> </ol> </li> <li>3. Transfection             <ol style="list-style-type: none"> <li>3.1 Prepare transfection mix A and B.                 <p>For per well of a 6-well plate: A: 120 <math>\mu</math>L serum-free medium + 5 <math>\mu</math>L miRNA mimics; B: 121 <math>\mu</math>L serum-free medium + 4 <math>\mu</math>L PolyFast Transfection Reagent (HY-K1014).</p> <p>For per well of a 24-well plate: A: 23.75 <math>\mu</math>L serum-free medium + 1.25 <math>\mu</math>L miRNA mimics; B: 24 <math>\mu</math>L serum-free medium + 1 <math>\mu</math>L PolyFast Transfection Reagent (HY-K1014).</p> <p>For per well of a 96-well plate: A: 4.75 <math>\mu</math>L serum-free medium + 0.25 <math>\mu</math>L miRNA mimics; B: 4.8 <math>\mu</math>L serum-free medium + 0.2 <math>\mu</math>L PolyFast Transfection Reagent (HY-K1014).</p> </li> </ol> <p>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,</p> </li> </ol> |

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  $\mu$ 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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