Natural miRNA Precursor miR-302bcad and miR-302bcad/367 Cell Extract and Purified

Protocol

Product Information

Natural microRNA Precursorsare microRNA transcripts that have not been processed into mature microRNA. Once in the cell of interest, they will be processed into mature microRNAs. These mature microRNAsperform the same functions and contain the same hairpin constructs seen in natural cellular counterparts. Natural microRNA Precursor facilitates research of authentic microRNA function. It does so by delivering natural microRNA function through a natural biogenesis mechanism. This will allow researchers the ability to study the cellular effects of microRNA on cell development, the overall effect of microRNA on gene silencing, as well as expand the boundaries of stem cell research.

Handling of Natural miRNA Precursors

Use RNAase-free agents, tubes, and pipette tips when handling this product. RNA oligonucleotides are susceptible to degradation by exogenous RNAases that can be introduced during handling. Always wear gloves when working with this product.

Storage of Natural miRNA Precursors in liquid

In liquid form, store this product at 4°C for up to 3 months or store below -80°C for one year. Repeated freezing and thawing actions will quickly degrade the precursors.

Potential applications of Natural miRNA Precursors

In order to better understand the role of Natural miRNA precursors in experimental applications, use a cell line that expresses low levels of the mature miRNA of interest.

Monitoring the effect on cellular processes

- Transfect a Natural miRNA Precursor into the cell line of interest.
- Compare the phenotype of the transfected cells to that of negative control-transfected cells. A change in cell morphology, viability, proliferation, signaling, metabolism, cell cycle, or any other of a variety of measurable phenotypes would indicate that the miRNA affects a cellular process.

Identifying endogenous miRNA target genes

1. Transfect a Natural miRNA Precursor into the cell line of interest.
2. Compare the expression of one or more putative target genes in Natural miRNA Precursor-transfected cells to that in negative control-transfected cells using, for example, one of the following analytical methods.
   - Protein targets: immunostaining, western analysis, or any of a variety of protein quantification methods.
   - mRNA targets: expression assays.

Guidelines for transfection

As with other small nucleic acids, such as siRNAs, the efficiency with which mammalian cells are transfected with Natural miRNA Precursor s depends on the cell type and transfection reagent used. Determine the optimal transfection conditions that result in maximum Natural miRNA Precursor-mediated activity with minimal cytotoxicity. Then:

- Maintain transfection conditions from experiment to experiment for a given cell type.
- Include controls in all plates for each experiment to ensure consistency.

Determine optimal transfection conditions

Use your experimental cell line and appropriate positive and negative control Natural miRNA Precursors. First, identify an effective transfection agent for your cell type. Then adjust accordingly:

a. Amount of transfection agent.
b. Amount of Natural miRNA Precursors.
c. Cell density at the time of transfection. In general, 30 to 70% confluency is recommended.
d. Transfection method.
e. Length of exposure of cells to transfection reagent-Natural miRNA Precursor complexes.

For Natural miRNA Precursors, maximal activity is achieved after 24 hours, and the existing medium can

be replaced with fresh medium 24 hours after transfection, resulting in greater viability of the cells.

Transfection concentrations

Natural miRNA Precursors typically work best when transfected at a final concentration of 200 µg/mL. Optimization experiments might include a concentration range of 100 µg/mL to 300 µg/mL.

Reference

1. Lin et al., (2010) MicroRNA miR-302 inhibits the tumorigenecity of human pluripotent stem cells by coordinate suppression of CDK2 and CDK4/6 cell cycle pathways. Cancer Res., 70: 9473-9482.
2. Lin et al., (2011) Regulation of somatic cell reprogramming through inducible mir-302 expression. Nucleic Acids Res., 39: 1054-1065.
3. Anokye-Danso et al., (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell, 8: 376-388.
4. Subramanyam D et al., (2011) Multiple targets of miR-302 and miR-373 promote reprogramming of humanfibroblasts to induced pluripotent stem cells. Nature Biotechnology., 29: 443-448.
5. Miyoshi N et al., (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell., 8: 633-638.

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