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Product Information

MISSION® Synthetic miRNA Inhibitors

Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

MISSION synthetic miRNA inhibitors (S-TuD)[1] are small, double-stranded RNA molecules designed to inhibit a specific mature microRNA (miRNA). When introduced into cells using a transfection reagent or electroporation, synthetic miRNA inhibitors can regulate gene expression in a variety of ways by binding its miRNA target in a sequence specific manner.

Each microRNA inhibitor is designed using a proprietary algorithm, developed in collaboration with Dr. Hideo Iba and Dr. Takeshi Haraguchi, University of Tokyo[1], using the mature miRNA sequence from the Sanger miRNA database (miRBase). The structure of these inhibitors is based upon the synthetic 'Tough Decoy' (TuD)[2] molecule. They are stabilized by incorporating 2'-O-methylated nucleotides, and are ready for transfection into mammalian cells.

Before transfecting with the miRNA inhibitor, Sigma strongly recommends verifying endogenous expression of the target miRNA in each cell type to be used by qRT-PCR or similar assay. We recommend using the MystiCq® microRNA cDNA Synthesis Mix kit and the MystiCq microRNA qPCR Assay Primers to quantitate specific endogenous miRNA levels. Starting with total RNA or RNA preparations pre-enriched for microRNAs, these kits provide the components necessary to convert mature microRNAs into cDNA templates for qPCR.

Once target miRNA expression has been verified in the cell line of choice, cells can be transfected with the miRNA inhibitors using the preferred transfection method for each cell type. After transfection, miRNA inhibition may be evaluated by a dual luciferase reporter assay, change in mRNA levels by qRT-PCR, microarray or RNA-sequencing, and/or change in protein levels by western blotting or mass spectrometry.

Product	Quantity*	Catalog Number
MISSION Synthetic miRNA Inhibitor, Human	5 nmol	HSTUDXXXX
MISSION Synthetic miRNA Inhibitor, Mouse	5 nmol	MSTUDXXXX
MISSION Synthetic miRNA Inhibitor, Custom	20 nmol	CSTUD
MISSION Synthetic miRNA Inhibitor, Negative Control 1 (*Arabidopsis thaliana*)	5 nmol	NCSTUD001
MISSION Synthetic miRNA Inhibitor, Negative Control 2 (*Caenorhabditis elegans*)	5 nmol	NCSTUD002

*Quantity provided is sufficient to bind 5 nanomoles of target miRNA. The custom option binds 20 nanomoles.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To resuspend the dried synthetic miRNA inhibitor, briefly spin the tube in a microcentrifuge before opening, then, according to Table 1, add water, Molecular Biology Reagent, e.g. Catalog Number W4502, to generate a stock solution of 10 µM. Mix by pipetting or vortexing. Once rehydrated, a 10 µM stock solution will have the following concentration: 10 mM potassium acetate, 3 mM HEPES-KOH, 0.2 mM magnesium acetate.

Table 1

Preparation of Synthetic miRNA inhibitors

Quantity of Synthetic miRNA inhibitor*	Volume of Water for 10 µM Stock Solution
5 nmol	500 µL
20 nmol	2 mL

*Quantity of target miRNA binding capacity

Storage/Stability
MISSION Synthetic miRNA Inhibitors are shipped dry at room temperature. Upon receipt, the synthetic miRNA inhibitor(s) should be stored at –20 °C. Stored under these conditions, MISSION Synthetic miRNA Inhibitors are guaranteed for 2 years dry or rehydrated. Store the synthetic miRNA inhibitor solutions in small aliquots at –20 °C and limit the number of freeze thaw cycles to <5.

Procedure

Transfection of MISSION Synthetic miRNA inhibitors
For optimal transfection efficiency, a range of synthetic miRNA inhibitor concentrations and cell densities should be tested. An initial starting concentration for optimization is 0.05-25 nM. Synthetic miRNA inhibitor concentrations of 0.005–50 nM have been used successfully in a variety of cell lines. Specific applications may require lower or higher concentrations to achieve desired inhibition levels.

Refer to the instructions provided with the transfection reagent or electroporation method. Known siRNA and other small RNA conditions can be used as a starting point for transfection optimization.

Assessing loss of miRNA function

Caution: The function of the miRNA should be assayed, not the presence or absence of miRNA. Mission Synthetic miRNA Inhibitors primarily act by binding and inhibiting miRNA activity, not by eliminating the miRNA.

miRNA inhibition may be evaluated by a number of different methods. Loss of miRNA function can be assessed by detecting an increase in target messenger RNA (mRNA) and/or protein. We recommend KiCqStart™ SYBR® Green Primers, Catalog Number KSPQ12012, and KiCqStart SYBR Green qPCR ReadyMixes, Catalog Numbers KCQS00, KCQS01, KCQS02 and KCQS03, to quantify specific mRNA targets. However, since miRNA targeting does not always lead to mRNA degradation, loss of miRNA function may require analysis of protein expression or activity. Reporter assays, such as a dual luciferase reporter assay with a miRNA target sequence inserted into the 3' untranslated region (3'UTR) after luciferase, may be used to detect loss of miRNA function. Alternatively, Western blot or other immunoassay and/or mass spectrometry may be used to investigate the impact of miRNA inhibition on protein quantity.

Reference

1. Haraguchi, T, *et al.* A potent 2'-O-methylated RNA-based microRNA inhibitor with unique secondary structures. Nucleic Acids Res. 2012 Apr;40(8):e58. Epub 2012 Jan 17.
2. Haraguchi, T., et al., Vectors expressing efficient RNA decoys achieve the long-term suppression of specific microRNA activity in mammalian cells. Nucleic Acids Res., 37, (2009).

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