SIGMA-ALDRICH®

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Product Information

MISSION® esiRNA

Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonym: Endoribonuclease-prepared siRNAs

Product Description
RNA interference (RNAi) is a cellular mechanism that decreases the function of a gene through the degradation of its associated messenger RNA (mRNA). The RNAi pathway is triggered by double-stranded RNA (dsRNA) complementary to the target gene mRNA. The most effective dsRNAs for RNAi in mammalian cells were found to be 18–25 bp in length with a 2-nt 3′ overhang (siRNA).[1] Chemically synthesized 21 bp siRNAs have been used successfully for loss-of-function studies in a large variety of different mammalian cell types as well as in whole organisms.[2] However, the efficiency and specificity of synthetic siRNAs can be low, a possible result of positional effects and the difficulty in choosing effective 21 bp sites in the target-gene mRNA.[3] The silencing effectiveness and specificity of an siRNA is highly dependent on the location of the corresponding target site.[4] In order to identify an siRNA sequence that optimally suppresses a target gene, siRNA sequences homologous to different regions of the target gene must be designed, synthesized, and tested, which can be time-consuming and expensive.

MISSION esiRNAs are an alternative way of accomplishing gene silencing by RNAi in mammalian cells. Endoribonuclease-prepared siRNAs (esiRNAs) are synthesized by *in vitro* transcription of a 300–600 bp gene specific dsRNA, followed by enzymatic digestion using RNAses (i.e., RNase III). This digest produces complex pools of siRNA-like molecules. All PCR products for esiRNA synthesis are verified by DNA sequencing and gel electrophoresis to guarantee identity. To ensure high specificity and efficacy of the esiRNA, the algorithm DEQOR is utilized (Design and Quality Control of RNAi, available to the public via http://cluster-1.mpi-bg.de/Deqor/deqor.html). This algorithm prioritizes the sites of the target mRNA by susceptibility, avoiding repetitive and redundant sequences.[5]

Because esiRNAs are pools of siRNAs that all target the same mRNA sequence, they are highly specific.[6] This strategy eliminates the trial and error approach of identifying a useful synthetic siRNA and ensures minimal risk of off-target effects. MISSION esiRNAs are powerful tools for gene silencing in mammalian cells.

Components
- All MISSION esiRNAs are delivered as frozen stocks resuspended in nuclease-free TE buffer (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA).
- Concentration is measured by OD_{260} or fluorescence intensity. Available sizes and concentrations are listed below:

Size (µg)	pmol*	Description	Package quantity	Concentration
1	~100	whole genome, non-coding and esiFLEX libraries	1 µg/esiRNA in library	50 ng/µL
2.5	~250	whole genome, non-coding and esiFLEX libraries	2.5 µg/esiRNA in library	50 ng/µL
5.0	~500	whole genome, non-coding and esiFLEX libraries	5.0 µg/esiRNA in library	50 ng/µL
20	~2000	Individual esiRNA, esiOPEN or esiSEC for a specific gene target	20 µg of the specified esiRNA	200 ng/µL
50	~5000	Individual esiRNA, esiOPEN or esiSEC for a specific gene target	50 µg of the specified esiRNA	200 ng/µL

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- **Please refer to Appendix 1 for more information on how to calculate the amount of esiRNA.**

- MiSSION esiRNA whole genome libraries are provided in 384-well plates.
- The Non-coding RNA esiRNA libraries are provided in 96-well plates.
- The standard plate format for both is all positions occupied.
- Individual, esiOPEN and esiSEC esiRNAs are supplied in tubes.
- esiFLeX esiRNAs allow the plate position of each esiRNA to be freely chosen.

MISSION esiRNAs are susceptible to degradation by nucleases introduced during handling. Use RNase-free reagents, tubes, and filtered/barrier pipette tips. Always wear gloves when handling MISSION esiRNA products.

Reagents Required but Not Provided
- Cell line of choice
- Nuclease-free water, Catalog Number W4502
- Transfection reagent/material for delivery
- Negative Controls (such as RnLuc, FfLuc, and EGFP esiRNAs)
- Positive Controls (such as KIF11 and Lamin-A/C esiRNAs)

Recommended Reagents, not Provided
- RNA isolation and purification reagents (such as GenElute™ Mammalian Total RNA Miniprep Kit, Catalog Numbers RTN10, RTN70, or RTN350)
- qPCR reaction components (such as SYBR® Green JumpStart™ *Taq* ReadyMix™, Catalog Number S4438)

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
Spin down the plate or tube before use, to ensure the solution is at the bottom. Dilutions should be performed in nuclease free TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). esiRNA should be kept on ice (4°C) during bench work.

Storage/Stability
MISSION esiRNAs are shipped on dry ice. For extended storage, freeze at −20 °C in working aliquots. At −20 °C, esiRNA remain stable for at least 2 years. Keep the working aliquots at a high concentration. Avoid storing esiRNAs at concentrations less than 20 ng/µL for longer than a few days. esiRNA concentrations of less than 20 ng/µL should only be done as working dilutions and are not suitable for storage.
Repeated freezing and thawing, or storage in "frostfree" freezers, is not recommended

Procedure
Experimental design
When conducting experiments using MISSION esiRNAs, proper controls are the key element for accurate interpretation of knockdown results. The MISSION esiRNA controls are useful for setting up experiments and for screening purposes using the libraries. MISSION esiRNAs against commonly used reporter genes may be used for optimization and/or transfection controls. These controls include *Renilla* luciferase, firefly luciferase, and EGFP. If the reporter gene is expressed in the cell line of interest, the controls can be used as positive controls by demonstrating knockdown after delivery. If the reporter gene is not expressed in the desired cell lines, the controls can be used as negative controls. When using these esiRNAs as positive controls, it is recommended to align the esiRNA sequence to the reporter cDNA to ensure homology. A full homology of the target-site and the esiRNA is required for proper silencing.

Catalog Number	Control description
EHURLUC	Renilla Luciferase
EHUFLUC	Firefly Luciferase
EHUEGFP	enhanced Green Fluorescent Protein
EHU019931	Human KIF11
EHU063791	Human LMNA-lamin A/C
EMU017691	Mouse Kif11
EMU056191	Mouse Lmna-lamin A/C

As positive controls, esiRNAs against the motor protein Eg5 (Kif11)[7] or the nuclear envelope protein, Lamin-A/C are recommended. Knockdown of Eg5 or Lamin-A/C decreases cell viability significantly. Therefore, both esiRNAs are suitable for measuring transfection efficiency as well as for optimization purposes in a large variety of human cell lines.

Recommended Controls
It is recommended to use the following controls for the MISSION esiRNA experiment:

- Non-transfected cells
- Transfection reagent only control
- Negative control esiRNAs (as previously listed)
- Positive control esiRNAs (as previously listed)

Ensure the cell line is healthy and the cells are at least 90% viable before transfection. To calculate the amount of esiRNAs, see Appendix 1.

Transfection of Mammalian Cells with MISSION esiRNA
MISSION esiRNAs arrive ready for transfection into the mammalian cell type of interest. See Appendix 2 for a list of cell lines that have been successfully transfected with esiRNAs. Transfect the MISSION esiRNA into the cell type of interest following the transfection reagent manufacturer's instructions. MISSION esiRNAs should be tested in a pilot experiment to validate the best concentration of esiRNAs for every new cell type and new experimental procedure. Known transfection conditions used for chemically synthesized siRNAs are a good starting point for optimization.

Optimizing Transfection of MISSION esiRNA
To obtain the highest transfection efficiency and lowest nonspecific effects, optimize transfection conditions by varying the cell density, the amounts of esiRNAs, and the transfection reagent as suggested by the supplier of the transfection reagent. A time course is recommended to identify the optimal point at which to detect the knockdown. Perform the appropriate assays to determine target gene suppression levels. When performing RNAi experiments using MISSION esiRNAs, researchers generally observe inhibition of the gene of interest within 24–96 hours after transfection. The degree of gene knockdown observed depends on factors such as the time of analysis, transcription rate of the gene of interest, protein half-life, and the growth characteristics of the mammalian cell line.

Monitoring Gene Silencing
To detect the silencing effect of the target MISSION esiRNAs, it is recommended to perform quantitative PCR (qPCR), in which the remaining amount of the transcript of interest is measured.

Caution: It is not recommended to use branched DNA technology for MISSION esiRNA experiments.

References

1. Elbashir, S.M. et al., Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, **411**(6836), 494 (2001).
2. Echeverri, C.J., and Perrimon, N., High-throughput RNAi screening in cultured cells: a user's guide. *Nat*. *Rev*. *Genet*., **7**(5), 373 (2006).
3. Jackson, A.L. et al., Expression profiling reveals off-target gene regulation by RNAi. *Nat*. *Biotechnol*., **21**(6), 635 (2003).
4. Holen, T. et al., Positional effects of short interfering RNAs targeting the human coagulation trigger Tissue Factor. *Nucleic Acids Res*., **30**(8), 1757 (2002).
5. Henschel, A. et al., DEQOR: a web-based tool for the design and quality control of siRNAs. *Nucleic Acids Res*., **32** (Web Server issue), W113 (2004).
6. Kittler, R. et al., Genome-wide resources of endoribonuclease-prepared short interfering RNAs for specific loss-of-function studies. *Nat*. *Methods*, **4**(4), 337 (2007).
7. Weil, D. et al., Targeting the kinesin Eg5 to monitor siRNA transfection in mammalian cells. *Biotechniques*, **33**(6), 1244 (2002).

Appendix 1
Calculation of esiRNA

The following information may be used to calculate the amount of esiRNAs. Since a high percentage of the esiRNA product is 20–22 bp, an average length of 21 bp can be used for the calculation. The average molecular mass of one base including sugar and phosphates is 345 g/mol.

For an esiRNA (ds) of 21 bp length the average molecular mass can be calculated:

MW = 2 × 21 × 345 = 14,490 g/mol or ~15 kDa

Appendix 2
Cell Lines

The listed cell types have been successfully transfected with esiRNAs.

Cell lines, human	**Cell Type**	**Transfection method**
HEK293	embryonic kidney cells	Lipofection
HeLa	cervical adenocarcinoma	Lipofection
HCT116	colon carcinoma	Lipofection
U2-OS	osteosarcoma	Lipofection
MV 4-11	leukemic	Electroporation
Cell lines, mouse	**Cell Type**	**Transfection method**
NIH 3T3	fibroblast	Lipofection
Primary cells	**Cell Type**	**Transfection method**
ECS	mouse embryonic stem cells	Lipofection
fibroblasts	mouse embryonic fibroblasts	Lipofection
Neuronal	mouse embryonic neuron precursor cells	Electroporation

Troubleshooting Guide

Problem	**Possible Cause**	**Suggestions**
Low levels of gene knockdown observed.	Low transfection efficiency	May be due to non-optimal transfection conditions or too little esiRNA. Monitor the transfection efficiency with labeled oligonucleotides. Try different transfection reagent/method.
	Assay performed too early.	Perform a time course study to assess the optimal point at which to harvest samples for subsequent knockdown analyses.
	Degraded esiRNAs	Aliquot esiRNAs upon arrival to avoid repeated freeze/thaw cycles.
Incidence of cell toxicity is high.	Too much transfection reagent used.	Perform a dose-response curve varying the amount of transfection reagent, while maintaining the amount of esiRNAs, to determine if too much reagent was used.
	Too much esiRNAs used.	Perform a dose-response curve varying the amount of esiRNAs used per sample.
	Transfection mixture is not evenly distributed.	Ensure transfection mixture is evenly distributed over all cells by gently rocking the dish back and forth, and side to side.
	Target gene is essential.	Assess viability in control wells, to see if toxicity is target gene-specific.
No gene knockdown observed.	Degraded esiRNAs	Aliquot esiRNAs upon arrival and avoid repeated freeze/thaw cycles.
	Problems with qPCR	Refer to the product troubleshooting guide for the qPCR reagent.

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