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Product Information

MISSION® esiRNA

Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonym: Endoribonuclease-prepared siRNA

Product Description
RNAi (RNA interference) is a cellular mechanism that silences genes via degradation of their associated mRNA (messenger RNA). RNAi is triggered by dsRNA (double-stranded RNA) complementary to the mRNA of the target gene. The most-effective dsRNA for RNAi in mammalian cells has been found to be 18 to 25 bp in length with 2-nt overhangs on the 3′ ends.[1] Chemically-synthesized 21 bp siRNA 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 siRNA can be low, a possible result of positional effects and the difficulty in choosing effective 21-bp sites in the mRNA of the target gene.[3,4] 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 esiRNA is an alternative way of accomplishing gene silencing via RNAi in mammalian cells. Endoribonuclease-prepared siRNA (esiRNA) are synthesized by *in vitro* transcription of a 300 to 600 bp gene-specific dsRNA, followed by enzymatic digestion using RNase (i.e., RNase III). This digest produces complex pools of siRNA with average lengths of 21 bp. All PCR products for esiRNA synthesis are verified by DNA sequencing and gel electrophoresis to guarantee identity. To ensure high specificity and efficacy of esiRNA, the DEQOR algorithm is utilized (RNAi design and quality control at http://144.76.155.9/deqor_new/input.html). DEQOR identifies sequences susceptible to significant silencing as well as avoids repetitive and redundant sequences.[5]

Because esiRNA are pools of siRNA that all target the same mRNA sequence, they are highly specific.[6] This strategy eliminates the trial and error approach of identifying a useful, chemically-synthesized siRNA and ensures minimal risk of off-target effects.

Components
- Products are delivered frozen (on dry ice) in nuclease-free TE buffer (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA).
- Available product options, descriptions, quantities, and concentrations are the following:

Option	Description	Quantity (µg)*	Concentration (ng/µL)
Individual esiRNA	Individual, predesigned** esiRNA for single protein-coding genes & lncRNA (long, non-coding RNA) in human and mouse.	20 & 50	200
esiLibrary	Predesigned esiRNA libraries for the whole genome & lncRNA transcriptome.	1, 1.5, & 5	50
esiFLEX	Custom arrays chosen from the whole genome & lncRNA transcriptome collections.	1, 2.5, & 5	50
esiOPEN	Custom esiRNA for single protein-coding genes & lncRNA.	20 & 50	200
esiSEC	Individual, secondary & independent esiRNA for validation of primary esiRNA targeting single protein-coding genes & lncRNA in human and mouse.	20 & 50	200

*See Appendix 1 to learn how to calculate the average molar mass of an esiRNA pool.
**Predesigned esiRNA are designed based on annotations from the ENSEMBL database.

- Depending on the nature of the library/array, esiLibrary and esiFLEX may come in 96 or 384 well plates with all wells occupied (well positions may be chosen with esiFLEX).
- Individual esiRNA, esiOPEN, and esiSEC are supplied in tubes.

EsiRNA are susceptible to degradation by nucleases introduced during handling. Use RNase-free reagents, tubes, filtered/barrier pipette tips, and gloves to prevent product loss.

Reagents Required but Not Provided.
- Cell line of choice
- Nuclease-free water (Catalog Number W4502)
- Transfection reagent/material for delivery
- Positive Controls, e.g. human Eg5/KIF11
- Negative Controls, e.g. RLUC, FLUC, and EGFP

Recommended Reagents but Not Provided
- RNA isolation and purification reagents, e.g. GenElute™ Mammalian Total RNA Miniprep Kit (Catalog Number RTN10, RTN70, or RTN350)
- qPCR reaction components, e.g. SYBR® Green JumpStart™ *Taq* ReadyMix™ (Catalog Number S4438)

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Centrifuge 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
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 esiRNA at concentrations <20 ng/µL for longer than a few days. EsiRNA concentrations of <20 ng/µL should only be used as working dilutions and are not suitable for storage. Repeated freezing and thawing, or storage in frost-free freezers, is not recommended.

Procedure
Experimental design
When conducting experiments using esiRNA, proper controls are the key element for accurate interpretation of knockdown results. EsiRNA controls are useful for setting up experiments and for screening purposes using the libraries. EsiRNA against commonly used reporter genes may be used for optimization and/or transfection controls. These controls include RLUC, FLUC, 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 esiRNA as positive controls, it is recommended to align the esiRNA sequence to the reporter cDNA to ensure homology. Complete homology of the target site and the esiRNA is required for proper silencing.

Figure 1.
Controls

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, esiRNA 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 esiRNA 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 an esiRNA experiment:

- Non-transfected cells
- Transfection reagent only control
- Negative control esiRNA (as listed above)
- Positive control esiRNA (as listed above)

Ensure the cell line is healthy and the cells are at least 90% viable before transfection (to calculate the molar mass of esiRNA, see Appendix 1).

Transfection of Mammalian Cells with esiRNA
EsiRNA arrive ready for transfection of the mammalian cell line of interest (see Appendix 2 for a list of cell lines that have been successfully transfected with esiRNA). Transfect the cell line of interest with esiRNA following the manufacturer's instructions for the transfection reagent. EsiRNA should be tested in a pilot experiment to validate the best concentration and experimental procedure to use with every cell line. Known transfection conditions used for chemically-synthesized siRNA are a good starting point for optimization.

Optimizing Transfection of esiRNA
To obtain the highest transfection efficiency and lowest nonspecific effects, optimize transfection conditions by varying the cell density, the amount of esiRNA, and the transfection reagent as suggested by the manufacturer 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 silencing levels. When performing RNAi experiments using esiRNA, researchers generally observe inhibition of the target gene within 24 to 96 hours post-transfection. The degree of silencing observed depends on factors such as the time of analysis, transcription rate, protein half-life, and the growth characteristics of the cell line.

Monitoring Gene Silencing
To detect the silencing, it is recommended to perform qPCR in which the remaining amount of the transcript of interest is measured.

Caution: It is not recommended to use branched DNA technology for 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 siRNA. *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).

Appendices
Appendix 1
Calculation of esiRNA molar mass

- A high percentage of the esiRNA pool is 21 bp
- The average molar mass of one base including sugar and phosphate is 345 g/mol.
- For an esiRNA (always double stranded) of 21 bp, the average molar mass can be calculated as follows:

$$2 \times 21 \times 345 = 14{,}490 \text{ g/mol}$$

Appendix 2
Cell Lines

These cell lines have been successfully transfected with esiRNA:

Cell Line	Origin	Plate Format	esiRNA Quantity (ng)	Transfection Reagent
Human cell lines				
HeLa	Human cervical carcinoma	96 well	30	Oligofectamine
U2OS	Human osteosarcoma	96 well	30	Oligofectamine, Effectene
HCT116	Human colorectal carcinoma	96 well	50	Oligofectamine
RKO	Human colorectal carcinoma	96 well	50	Oligofectamine
SW48	Human colorectal carcinoma	96 well	40	Oligofectamine
DLD-1	Human colorectal carcinoma	96 well	50	Lipofectamine 2000
HEK293	Human embryonic kidney	384 well	25	Effectene
Mouse cell lines				
NIH3T3	Immortalized mouse embryonic fibroblasts	96 well	200	Lipofectamine RNAi/MAX
Primary cell lines				
R1/E	129v mouse ES	96 well	50	Lipofectamine 2000
E14Tg2a	129v mouse ES	96 well	50	Lipofectamine 2000

Troubleshooting Guide

Problem	Possible Causes	Suggestions
Low level of gene knockdown	Low transfection efficiency	Re-evaluate the transfection reaction. Perform a dose-response curve to optimize the amount of transfection reagent and esiRNA. Use positive controls to investigate knockdown efficiency. Use negative controls to identify cytotoxic effects due to high concentrations of transfection reagent and esiRNA.
	Assay performed too early	Perform phenotypic analysis of cells transfected with positive controls.
	Degraded esiRNA	Aliquot esiRNA upon arrival to avoid repeated freeze/thaw cycles.
High cell toxicity	Too much transfection reagent used	Perform a dose-response curve varying the amount of transfection reagent, while maintaining the amount of esiRNA, to determine if too much reagent was used.
	Too much esiRNA used	Perform a dose-response curve varying the amount of esiRNA 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	Degraded esiRNA	Aliquot esiRNA 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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