B RNAi

siRNA Synthesis Service
miRNA Synthesis Service
In vivo siRNA Custom Service: SAMiRNA™

Life Science Product

01

siRNA Synthesis Service

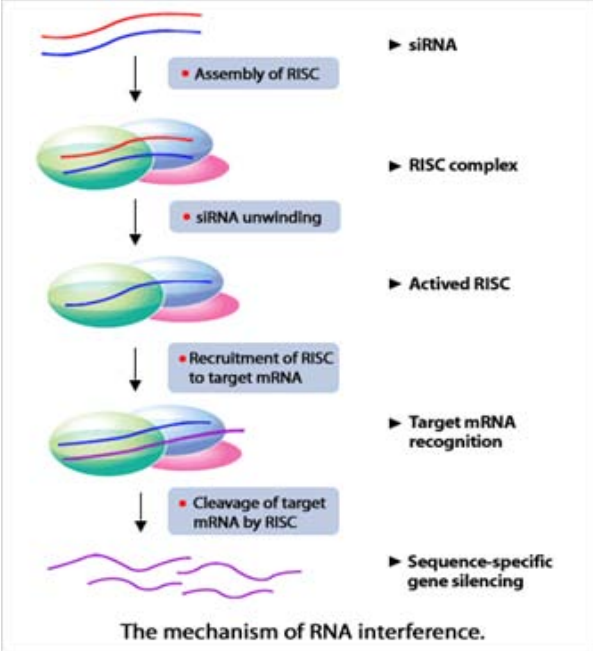
Overview

Gene knockdown technologies, such as antisense, ribozyme, and gene knockouts are used to perform loss-of-function studies. However, the post-genomics era calls for high-throughput gene function studies, at which the prior gene knockdown approach are provide due to poor reproducibility, high cost, and excessive time to the result. Thus, the advent of short interfering RNA or siRNA technology has opened up many new possibilities in the field of gene suppression. siRNA has the following advantages over other RNAi technology:

- Reduced time and costs: Less screening is required to obtain highly effective siRNA.
- High efficacy at lower concentration: Lower concentrations provide effective gene silencing and minimizes off-target effects.
- Specificity: siRNA is a highly specific target knockout mechanism based on the natural biological mechanisms of RNAi.

siRNA mechanism

A siRNA consists of 20 - 25-base pair RNA duplexes, where the two terminal 3'-nucleotides are unpaired (3'-overhang). When siRNAs are introduced into cells they combine with a protein complex called the RNA-induced silencing complex (RISC) and are unwound by a helicase. The RISC complex containing single-stranded RNA complementary to the target mRNA then recognizes and binds to the target mRNA. After binding the mRNA, the argonaute protein (Ago2) cleaves it and degrades the target mRNA completely via ribonuclease activity, based on the lack of protection by 5' caps or poly (A) tails). This method is most effective for the gene silencing of specific target genes, essential for gene function validation studies, drug target validation, and for gene therapy studies.



The mechanism of RNA interference.

Turbo si-Designer - Bioneer's proprietary siRNA design algorithm

RNA interference (RNAi) is a mechanism of gene silencing at the mRNA level. This phenomenon is triggered by small interfering RNA (siRNA) and micro RNA (miRNA). These molecules involved in gene regulation belong to an expanding class of small non-coding RNAs. siRNA is capable of inhibiting gene expression by either directing the degradation of homologous mRNA targets or inducing the repression of translation of mRNA targets.

In 2002, siRNA was hailed by Science magazine as being the "Breakthrough of the year" technology. In RNAi experiments, the most critical design factor is specific target recognition which is critical because the efficiency level of siRNA is different for each site. Silencing at the correct region of gene enables researchers to obtain reproducible experimental results which can lead to the subsequent use of siRNA as a genetic drug. Experimental success depends upon several factors.

The most critical among these factors is the design of effective and specific siRNA. Bioneer, in collaboration with the world renowned National Genome Information Center (NGIC) at KRIBB institute, has developed Turbo si-Designer, which is a proprietary siRNA selection algorithm. Turbo si-Designer identifies highly effective siRNA target sites with exceptional success rates. Several important parameters including base composition, the number of repetitive bases in a row, thermodynamic instability, energy profiling and base preference were considered in the development of Turbo si-Designer. The siRNAs spanning SNP sites are removed and non-specific siRNAs are eliminated after BLAST to minimize off-target effects. The resulting candidates are then ranked according to the NGIC scoring system. The performance of the algorithm was evaluated by designing hundreds of siRNAs and testing the siRNA knockdown efficacy by Real-Time PCR analysis. Over 80% of the siRNAs tested showed >75% knockdown of the target mRNA and more than 40% of siRNAs induced >90% knockdown. Notably, siRNAs with the low NGIC score were mostly nonfunctional, indicating that ineffective siRNAs are efficiently removed by Turbo si-Designer.

As one example to validate the performance efficacy of Turbo si-Designer, Bioneer tested the knockdown efficiency of 82 predesigned siRNAs for Survivin, which is one of anti-apoptosis and cell division related genes. Those siRNAs were transfected into A549 lung carcinoma cells and the knockdown efficiency was then analyzed using QuantiGene ViewRNA Analysis. As seen on the Figure 1, the lower-scoring siRNAs are not effective compared to the higher-scoring siRNA (Figure 1A and 1B.) and thus Turbo si-Designer can predict the higher efficiency siRNA by the exclusion of ineffective siRNA sites.

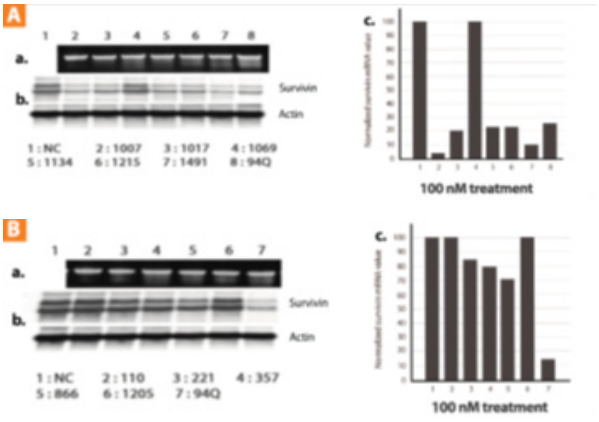


Figure 1. siRNA knockdown efficiency of siRNAs designed by Turbo si-Designer was analyzed by Northern blot and Real-Time PCR analysis.

A) siRNA knockdown efficiency of high NGIC score siRNAs.

B) siRNA knockdown efficiency of low NGIC score siRNAs.

AccuTarget™ Genome-wide Predesigned siRNA Library

Description

Bioneer offers over 132,000 predesigned siRNAs for more than 44,000 target genes from Human, Mouse and Rat. Search our extensive siRNA library by Gene ID, Symbol, Synonyms, Description, or Accession Number. Once you find your gene of interest, choose your guaranteed yield and purification level, and check your predicted siRNA knockdown efficiency. You can even order your qPCR primers for siRNA knockdown validation. Convenient and easy siRNA ordering - only from Bioneer.

The Bioneer Guarantee

When purchasing 3 siRNAs for the same gene, Bioneer guarantees at least an 80% reduction in the target mRNA level for two of the siRNAs. If there is not a >80% reduction in the mRNA level of the target gene, Bioneer will provide a replacement of 2 siRNAs free of charge. Bioneer reserves the right to request supporting data inclusive of:

1. siRNA Knockdown efficiency data: NC (*AccuTarget*™ Negative Control) and customer's siRNA concentration at 100 nM, respectively
2. Transfection efficiency data: PC (*AccuTarget*™ Positive control siRNA) and NC (*AccuTarget*™ Fluorescein-labeled Negative Control) siRNAs, respectively

Features and Benefits

- High siRNA knockdown rates:

  Two of three siRNAs suppress target mRNA levels >80% (Figure 2)

- Unique design algorithm:

  Maximized siRNA knockdown while minimizing off target effects.

- Competitive pricing:

  Great value for your research dollar

Application

AccuTarget™ Genome-wide Predesigned siRNA Library can be used in a variety of RNAi experiments.

- Functional genomics and proteomics research
- Gene expression studies
- Array analysis

Ordering Information

Purification	Guaranteed nmole
Bio-RP	10 nmole
	20 nmole
	50 nmole
	100 nmole
HPLC	10 nmole
	20 nmole
	50 nmole
	100 nmole

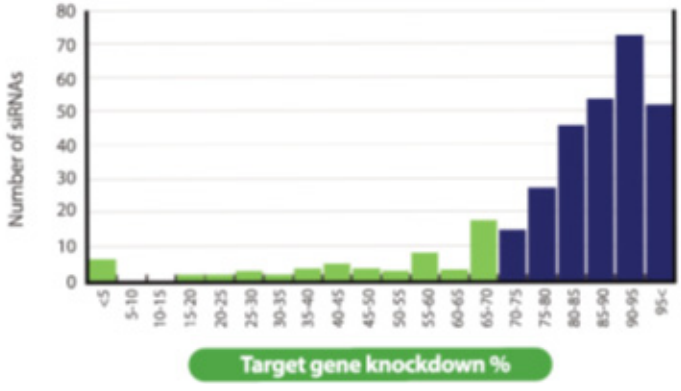


Figure 2. siRNA knockdown efficiency of *AccuTarget*™ Genome-wide Predesigned siRNA.

AccuTarget™ Predesigned siRNAs are highly effective. To determine siRNA knockdown efficiency of predesigned siRNAs, HeLa cells were transfected with siRNAs at 100 nM concentration. 24 hr post-transfection, total RNA was isolated and the level of target mRNA was measured by qRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm: 83.8% of tested siRNAs induced >70% siRNA knockdown and 38.1% of tested siRNAs elicited >90% knockdown.

AccuTarget™ Real-Time PCR Primer Library

Description

AccuTarget™ Human Validated Real-Time PCR Primer Library Set is comprised of highly specific and sensitive Real-Time PCR primer Sets that are bioinformatically designed and validated based on the human genome. The Real-Time PCR Primer Library consists of 11,154 primer Sets validated in Real-Time PCR with SYBR Green detection using

This *AccuTarget*™ Human Validated Real-Time PCR Primer Library Set is categorized by gene function and pathway. The Library guarantees the most specific and sensitive Real-Time PCR result (Figure 3) when used with *AccuPower® GreenStar*™ qPCR PreMix.

Features and Benefits

- In ready-to-ship format:
  11,154 human genes specific primers
- All Primers pre-validated
- Competitive pricing:
  Great value for your research dollar

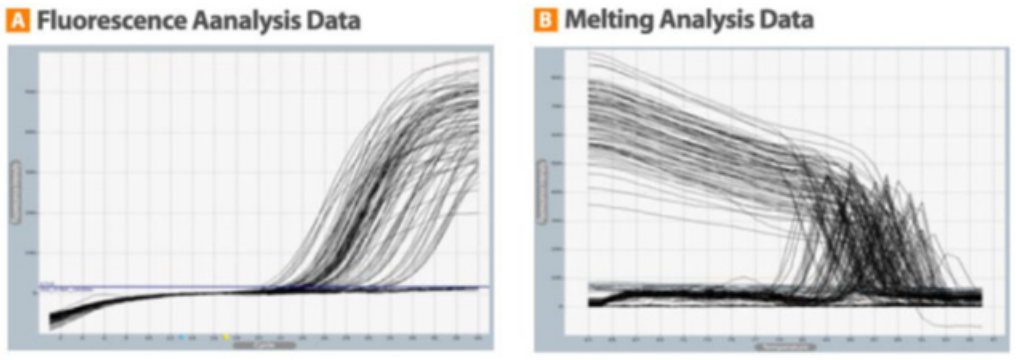


Figure 3. Real-Time PCR validation test of human oxidoreductase using *AccuTarget*™ Human Oxidoreductase Real-Time PCR Primer set

Ordering Information

AccuTarget™ Real-Time PCR Primer for Individual Gene

Product Description	Reactions
AccuTarget™ Human Real-Time PCR Primer for Individual Gene	100 rxns
AccuTarget™ Human Real-Time PCR Primer for Individual Gene	200 rxns

Description

AccuTarget™ Positive control siRNA are designed to induce high siRNA knockdown of their target genes. (Figure 4, 5 & 6). siRNA targeting an endogenous gene (GAPDH) and a reporter system (GFP and Luciferase) are available. *AccuTarget*™ Negative Control siRNAs do not target any known genes in human, mouse and rat. The negative control siRNA can be fluorescently labeled for easier monitor of transfection efficiency. *AccuTarget*™ Control siRNA Sets consisting of a positive and negative control siRNAs are also available for user convenience (Figure 7).

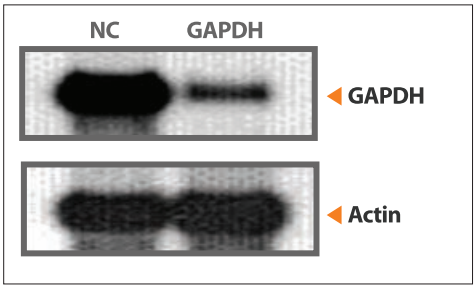
Features and Benefits

- Excellent performance:
  Positive control siRNA knockdown rates >90%
- Monitoring of transfection rate:
  Convenient fluorescently labeled negative control sets
- Competitive pricing:
  Great value for your research dollar

Positive control siRNA

1. GAPDH–siRNA

A) Northern blotting



B) RT-qPCR

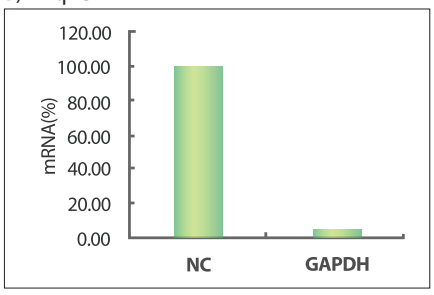


Figure 4. Effects of Human GAPDH Positive Control siRNA.

HeLa cells were transfected separated with *AccuTarget*™ Human GAPDH Positive Control and Negative Control siRNA using lipofectamine 2000 (Invitrogen) at a final concentration of 100 nM. Total cellular RNA was isolated from transfected cells 24 hr after transfection and subjected to northern blot and Real-Time PCR analysis. As can be seen from Figure 4-B, about 3% GAPDH mRNA remained.

2. GFP–siRNA

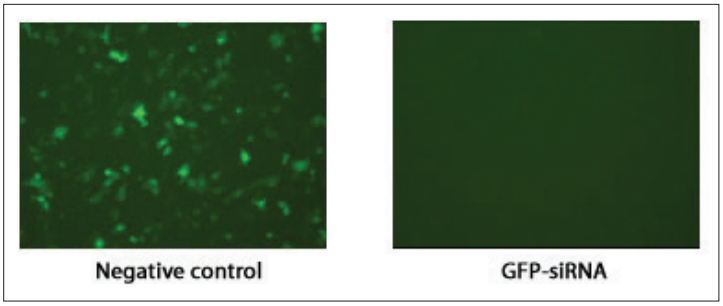


Figure 5. HeLa cells in a 24-well plate were co-transfected with 200 ng of CMV-GFP plasmid and 10 nM of GFP siRNA using lipofectamine 2000 transfection reagent.

Next day, the expression of GFP was observed by using a Nikon Eclipse TS100 epifluorescence microscope. In contrast to bright green fluorescence of GFP protein in NC-siRNA-transfected cells, no fluorescence was detected from GFP-siRNA-transfected cells, indicating efficient knockdown of GFP by using our positive control GFP-siRNA.

3. Luciferase-siRNA

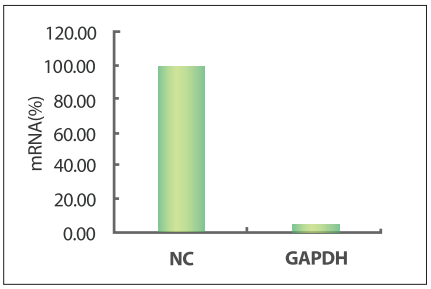


Figure 6. HeLa cells in a 6-well plate were co-transfected with 400 ng of CMV-luc plasmid and 10 nM of luciferase siRNA using lipofectamine 2000 transfection reagent. Next day, cells were harvested and assayed for luciferase activity. As shown in Figure. 6, co-transfection with our positive control luciferase siRNA led to efficient knockdown of luciferase activity (85% - 95% knockdown compared to luciferase activity of NC-siRNA-transfected cells).

4. Mouse Positive control siRNA

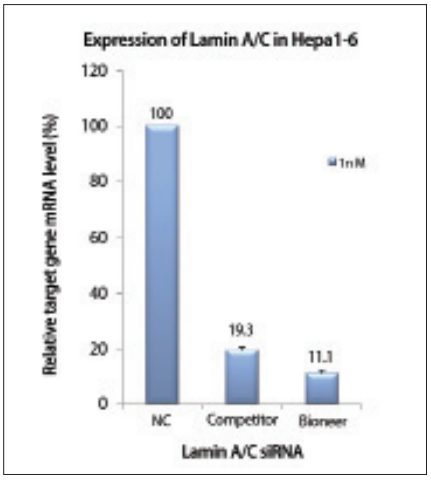


Figure 7. Effects of Mouse Lamin A/C Positive Control siRNA.

Hepa1-6 cells were transfected separately with *AccuTarget*™ Mouse Lamin A/C Positive Control and Negative Control siRNA using Lipofectamine RNAimax (Invitrogen) at a final concentration of 1 nM. Total cellular RNA was isolated from transfected cells 24 hr after transfection and subjected to Real-Time PCR analysis

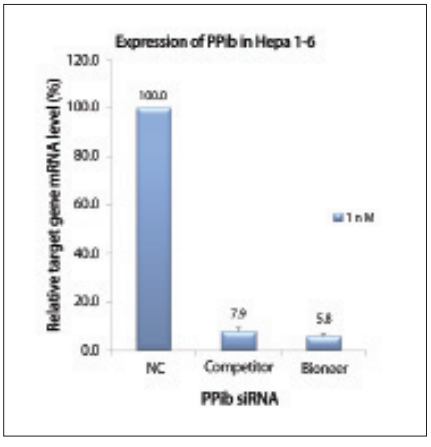


Figure 8. Effects of Mouse PPib (Cyclophilin B) Positive Control siRNA.

Hepa 1-6 & NIH3T3 cells were transfected separately with *AccuTarget*™ Mouse PPib (Cyclophilin B) Positive Control and Negative Control siRNA using Lipofectamine RNAimax (Invitrogen) at a final concentration of 1 nM. Total cellular RNA was isolated from transfected cells 24 hr after transfection and subjected to Real-Time PCR analysis.

Negative Control siRNA

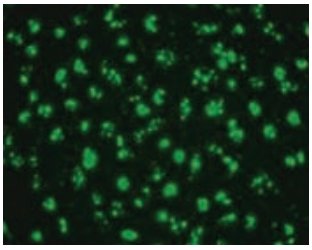


Figure 9. HeLa cells transfected with FITC-labeled siRNA (Cat. no. SN-1021) was observed by confocal microscopy. The fluorescent cells indicate that the target cells were successfully transfected with the siRNA.

Ordering Information

Cat. no.	Description	Purification	Guaranteed Yield
SP-1001	*AccuTarget*™ GAPDH Positive Control siRNA	Bio-RP	5 nmole
SP-1002	*AccuTarget*™ GAPDH Positive Control siRNA	Bio-RP	10 nmole
SP-1003	*AccuTarget*™ GAPDH Positive Control siRNA	Bio-RP	20 nmole
SP-1011	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	5 nmole
SP-1012	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	10 nmole
SP-1013	*AccuTarget*™ GAPDH Positive Control siRNA	HPLC	20 nmole
SP-2001	*AccuTarget*™ GFP Positive Control siRNA	Bio-RP	5 nmole
SP-2002	*AccuTarget*™ GFP Positive Control siRNA	Bio-RP	10 nmole
SP-2003	*AccuTarget*™ GFP Positive Control siRNA	Bio-RP	20 nmole
SP-2011	*AccuTarget*™ GFP Positive Control siRNA	HPLC	5 nmole
SP-2012	*AccuTarget*™ GFP Positive Control siRNA	HPLC	10 nmole
SP-2013	*AccuTarget*™ GFP Positive Control siRNA	HPLC	20 nmole
SP-3001	*AccuTarget*™ Luciferase Positive Control siRNA	Bio-RP	5 nmole
SP-3002	*AccuTarget*™ Luciferase Positive Control siRNA	Bio-RP	10 nmole
SP-3003	*AccuTarget*™ Luciferase Positive Control siRNA	Bio-RP	20 nmole
SP-3011	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	5 nmole
SP-3012	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	10 nmole
SP-3013	*AccuTarget*™ Luciferase Positive Control siRNA	HPLC	20 nmole
SP-4001	*AccuTarget*™ Mouse Lamin A/C Positive Control	Bio-RP	5 nmole
SP-4002	*AccuTarget*™ Mouse Lamin A/C Positive Control	Bio-RP	10 nmole
SP-4003	*AccuTarget*™ Mouse Lamin A/C Positive Control	Bio-RP	20 nmole
SP-4011	*AccuTarget*™ Mouse Lamin A/C Positive Control	HPLC	5 nmole
SP-4012	*AccuTarget*™ Mouse Lamin A/C Positive Control	HPLC	10 nmole
SP-4013	*AccuTarget*™ Mouse Lamin A/C Positive Control	HPLC	20 nmole
SP-5001	*AccuTarget*™ Mouse cyclophilin B Positive Control	Bio-RP	5 nmole
SP-5002	*AccuTarget*™ Mouse cyclophilin B Positive Control	Bio-RP	10 nmole
SP-5003	*AccuTarget*™ Mouse cyclophilin B Positive Control	Bio-RP	20 nmole
SP-5011	*AccuTarget*™ Mouse cyclophilin B Positive Control	HPLC	5 nmole
SP-5012	*AccuTarget*™ Mouse cyclophilin B Positive Control	HPLC	10 nmole
SP-5013	*AccuTarget*™ Mouse cyclophilin B Positive Control	HPLC	20 nmole

AccuTarget™ Control siRNAs

AccuTarget™ Negative Control siRNAs

Cat. no.	Description	Purification	Guaranteed Yield
SN-1001	*AccuTarget*™ Negative Control siRNA	Bio-RP	5 nmole
SN-1002	*AccuTarget*™ Negative Control siRNA	Bio-RP	10 nmole
SN-1003	*AccuTarget*™ Negative Control siRNA	Bio-RP	20 nmole
SN-1011	*AccuTarget*™ Negative Control siRNA	HPLC	5 nmole
SN-1012	*AccuTarget*™ Negative Control siRNA	HPLC	10 nmole
SN-1013	*AccuTarget*™ Negative Control siRNA	HPLC	20 nmole
SN-1021	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	5 nmole
SN-1022	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	10 nmole
SN-1023	*AccuTarget*™ Fluorescein-labeled Negative Control siRNA	HPLC	20 nmole

AccuTarget™ Control siRNA Sets

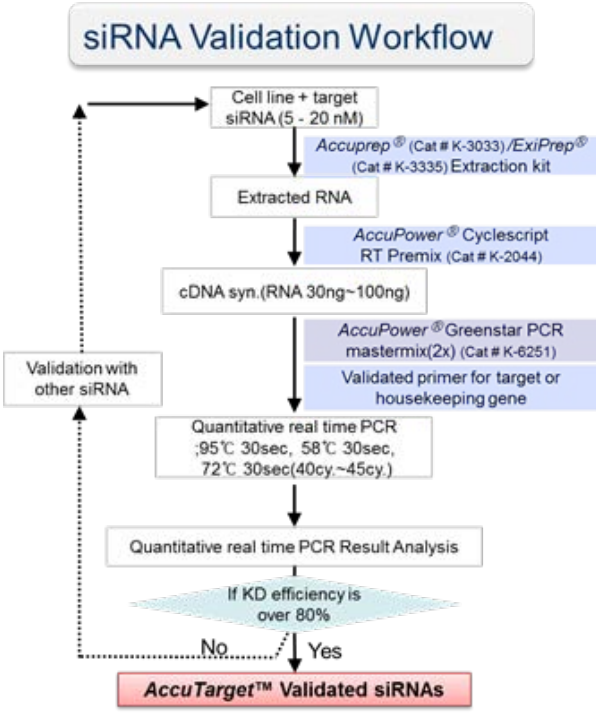
Cat. no.	Description	Purification	Guaranteed Yield
SS-1001	*AccuTarget*™ GAPDH Control siRNA Set	Bio-RP	5 nmole positive control + 2 nmole negative control
SS-1002	*AccuTarget*™ GFP Control siRNA Set	Bio-RP	5 nmole positive control + 2 nmole negative control
SS-1003	*AccuTarget*™ Luciferase Control siRNA Set	Bio-RP	5 nmole positive control + 2 nmole negative control
SS-1011	*AccuTarget*™ GAPDH Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control
SS-1012	*AccuTarget*™ GFP Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control
SS-1013	*AccuTarget*™ Luciferase Control siRNA Set	HPLC	5 nmole positive control + 2 nmole negative control

01 *AccuTarget*™ Premade siRNA Sets

Description

Bioneer offers the *AccuTarget*™ Premade siRNA Sets, which contain 25,368 predesigned and manufactured siRNAs for immediate use in your experiments. These Premade human siRNA Sets are available at 10, 20, 50 and 100 nmole guaranteed yield. We also offer 25 Pathway-specific / gene family siRNA Sets for researchers studying cellular processes, cancer, and disease etc. These are available at 0.1, 0.25, 0.5 and 1 nmole guaranteed yield. Finally there are 21 pre-validated siRNA libraries with high knockdown rates and demonstrated effectiveness. Validated siRNAs can be ordered at 10, 20, 50 and 100 nmole guaranteed yield.

Validation process of our designed siRNA is as follows:



Features and Benefits

- Categorized by Pathway/family:
  Convenient format for research
- Pre-validated siRNA libraries available:
  Works the first time and every time.
- Competitive pricing:
  value for your research dollar

Application

- *AccuTarget*™ Premade siRNA Sets can take advantage of the various RNAi experiments
- Pathway analysis and target identification and validation
- Drug target HTS (High Throughput siRNA Screening)

Ordering Information

AccuTarget™ Premade Human siRNA sets					
Cat. no.	Product Name	No. of Genes	Cat. no.	Product Name	No. of Genes
SHS-001	Antioxidant siRNA set	38	SHS-013	Lyase siRNA set	123
SHS-002	Apoptosis siRNA set	290	SHS-014	Motor siRNA set	122
SHS-025	Cancer siRNA set	1157	SHS-015	NF-kB pathway siRNA set	37
SHS-003	Caspase siRNA set	37	SHS-016	Nucleic acid binding siRNA set	2573
SHS-004	Cell cycle siRNA set	112	SHS-017	Oxidoreductase siRNA set	551
SHS-005	Cyclase siRNA set	21	SHS-018	Peptidase siRNA set	491
SHS-006	Cytochrome P450 siRNA set	52	SHS-019	Phosphatase siRNA set	188
SHS-007	Deaminase siRNA set	22	SHS-020	Receptor siRNA set	1516
SHS-008	GPCR signaling pathway siRNA set	727	SHS-021	Transferase siRNA set	1428
SHS-009	Helicase siRNA set	114	SHS-022	Transporter siRNA set	1021
SHS-010	Isomerase siRNA set	104	SHS-023	Tubulin siRNA set	20
SHS-011	Kinase siRNA set	699	SHS-024	Ubiquitin siRNA set	77
SHS-012	Ligase siRNA set	272			

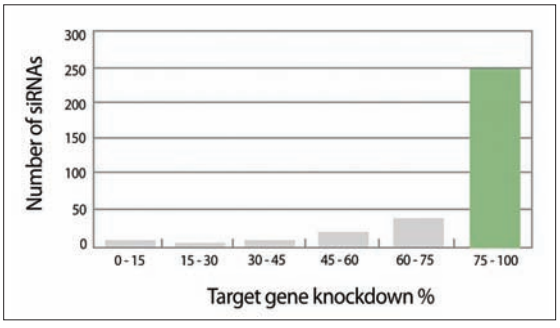


Figure 10. Knockdown efficiency of *AccuTarget*™ siRNA Library

To determine knockdown efficiency, HeLa cells were transfected with individual siRNAs at 100 nM concentration. 24 hr post-transfection, total RNA was isolated and the level of target mRNA was measured by qRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm: 83.8% of tested siRNAs induced >70% knockdown and 38.1% of tested siRNAs elicited >90% knockdown.

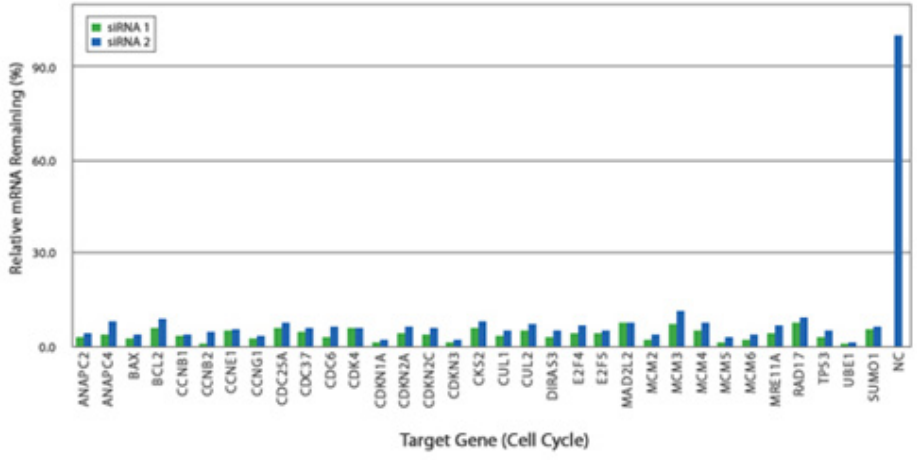


Figure 11. siRNA knockdown efficiency of each siRNA from *AccuTarget*™ Human Validated Cell cycle siRNA Set.

HeLa cells were transfected with siRNAs targeting 33 different genes at a concentration of 20 nM. Total RNA was isolated and the level of target mRNA was measured by qRT-PCR. This data demonstrates the effectiveness of the Turbo si-Designer algorithm.

AccuTarget™ Real-Time PCR Primers for Premade siRNA Sets

Description

AccuTarget™ Real-Time PCR Primer Sets are consisted of 25 groups of primer sets for Premade siRNAs categorized by pathway/gene function. These sets are optimized for real-time PCR using SYBR Green (*AccuPower*® *GreenStar*™ qPCR PreMix, Cat. no. K-6210) in order to get more specific and sensitive result.

Ordering Information

Product Description	No. of Genes	Reactions / gene
AccuTarget™ Human Antioxidant Real-Time PCR primer Set	38	50 rxns
AccuTarget™ Human Apoptosis Real-Time PCR primer Set	277	50 rxns
AccuTarget™ Human Cancer Real-Time PCR primer Set	1,082	50 rxns
AccuTarget™ Human Caspase Real-Time PCR primer Set	35	50 rxns
AccuTarget™ Human Cell cycle Real-Time PCR primer Set	111	50 rxns
AccuTarget™ Human Cyclase Real-Time PCR primer Set	20	50 rxns
AccuTarget™ Human Cytochrome P450 Real-Time PCR primer Set	37	50 rxns
AccuTarget™ Human Deaminase Real-Time PCR primer Set	19	50 rxns
AccuTarget™ Human GPCR signaling pathway Real-Time PCR primer Set	566	50 rxns
AccuTarget™ Human Helicase Real-Time PCR primer Set	112	50 rxns
AccuTarget™ Human Isomerase Real-Time PCR primer Set	91	50 rxns
AccuTarget™ Human Kinase Real-Time PCR primer Set	673	50 rxns
AccuTarget™ Human Ligase Real-Time PCR primer Set	261	50 rxns
AccuTarget™ Human Lyase Real-Time PCR primer Set	118	50 rxns
AccuTarget™ Human Motor Real-Time PCR primer Set	111	50 rxns
AccuTarget™ Human NF-kB pathway Real-Time PCR primer Set	37	50 rxns
AccuTarget™ Human Nucleic acid binding Real-Time PCR primer Set	2,235	50 rxns
AccuTarget™ Human Oxidoreductase Real-Time PCR primer Set	502	50 rxns
AccuTarget™ Human Peptidase Real-Time PCR primer Set	461	50 rxns
AccuTarget™ Human Phosphatase Real-Time PCR primer Set	179	50 rxns
AccuTarget™ Human Receptor Real-Time PCR primer Set	1,288	50 rxns
AccuTarget™ Human Transferase Real-Time PCR primer Set	1,355	50 rxns
AccuTarget™ Human Transporter Real-Time PCR primer Set	946	50 rxns
AccuTarget™ Human Tubulin Real-Time PCR primer Set	11	50 rxns
AccuTarget™ Human Ubiquitin Real-Time PCR primer Set	70	50 rxns

Description

Bioneer's Custom siRNA synthesis service offers exceptional quality siRNAs to knock down your target genes. Custom siRNAs can be synthesized according to sequence information you provide, or you can take advantage of our complimentary siRNA design service*. Up to 30-mer siRNA including a choice of 32 different 3' overhangs can be ordered with a variety of modification options for expanded specificity. siRNA is provided purified, annealed, lyophilized and ready-to-use. For even greater convenience, check out our AccuTarget™ Genome-wide Pre-designed siRNA - siRNAs pre-designed for human (18,048 genes), mouse (17,117 genes) and rat (8,392 genes) synthesized and ready-to-ship.

All custom siRNAs are synthesized in our state-of-the-art clean room facility and then purified free of charge utilizing Bioneer's Bio-RP purification system. For higher purity, HPLC purification is available at an additional charge. Each siRNA is quality controlled by MALDI-TOF mass spectrometry to guarantee highest quality (Figure 1), and analyzed by PAGE to confirm its duplex structure (Figure 2).

Features and Benefits

- Guaranteed performance:

  Two of three custom siRNA will give 80% siRNA knockdown**
- Guaranteed quality:

  Manufactured in a state-of-the-art clean room and QC'ed by MALDI-TOF and PAGE
- Custom siRNA design service available:

  Turbo si-Designer software design is available free of charge*.
- Competitive pricing:

  Overhang and annealing service provided free of charge, great value for your research dollar.

* Complimentary siRNA design service is available only when a customer orders the corresponding siRNA(s) from Bioneer.

** 80% siRNA knockdown guarantee policy is not applied to custom siRNA design for noncoding RNA by Turbo si-Designer.

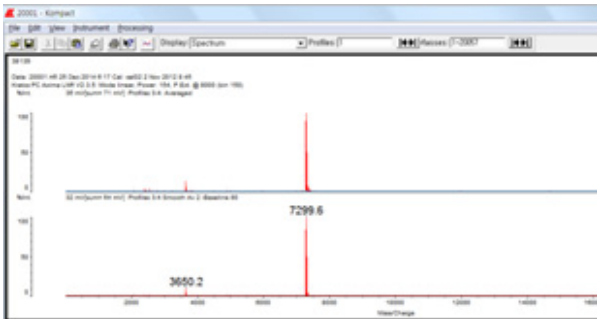


Figure 1. MALDI-TOF mass spectrometry analysis of a custom siRNA. All siRNAs are subjected to MALDI-TOF mass spectrometry to ensure its quality.

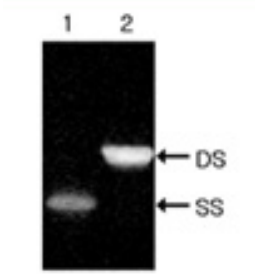


Figure 2. PAGE data of annealed double-stranded custom siRNA. Complementary single-strand RNA strands were hybridized to form siRNA duplex and analyzed by 15% non-denaturing PAGE.

SS: single-strand RNA, DS: double-strand siRNA.

Ordering Information

- Synthesis/Purification

Cat. no.	Purification	Guaranteed nmole
S-1017-1	Bio-RP	10 nmole
S-1017-2		20 nmole
S-1017-3		50 nmole
S-1017-4		100 nmole
S-1018-1	HPLC	10 nmole
S-1018-2		20 nmole
S-1018-3		50 nmole
S-1018-4		100 nmole

✓siRNA provided lyophilized and annealed.

✓Annealing buffer is provided when single-stranded siRNA requested

Modification available for custom siRNA

5'- Modification	3'- Modification	Internal Modification	Purification	Guaranteed Yield
5' Fluorescein	3' Fluorescein	Phosphorothioate	HPLC	10 nmole, 20 nmole 50 nmole 100 nmole
5' Phosphorylation	3' Phosphorylation	2'-OMe-rA, rC, rG, rU		
5' Biotin	3' Biotin	2'-F(A)		
5' Amine	3' Amine	2'-F(U)		
5' TAMRA	3' TAMRA	2'-F(G)		
5' Thiol	3' Thiol	2'-F(C)		
5' Cy3	3' DABCYL	Inosine		
5' Cy5	3' Cholesterol	Deoxy-abase		
5' PEG 2000	3' PEG 2000	Chimeric DNA		
5′ Cy5.5	5′ Cy5.5			

✓Certain items may not be available in all countries.

Contact Us

E-mail: siRNA@bioneer.co.kr

siRNA Oligonucleotide FAQs

1. What form will my order be in?
For Genome-Wide Predesigned siRNAs, Validated siRNAs, siRNA Libraries and Control siRNAs, both the sense and antisense strands are synthesized at equimolar concentrations, verified via MALDI-TOF, then annealed and delivered in duplexed, lyophilized form. You may reconstitute the siRNA with a buffer of your choice or with ultrapure water that we provide with every order. We recommend reconstituting to 100 µm. For Custom siRNA orders, the order is processed by the same method as above. We recommend 50 µM reconstitution for Custom siRNA orders. When ordering Custom siRNA, you must select the "Annealing Service" to receive your order in annealed form. If you choose not to use our annealing service, you can use 1X annealing buffer and follow the annealing protocol included with your Custom siRNA order

2. I want to conduct an *in vitro* experiment. What scale should I choose? What purification should I select?
With a 10 nmole scale siRNA order, you can transfect one hundred (100) wells in 6-well plates at 100 nm per transfection. Unless you are planning to conduct an in vivo experiment, the Bio-RP Purification will yield outstanding results. We recommend HPLC purification for in vivo experimental use.

3. *AccuTarget*™ Genome-wide predesigned siRNA didn't work like I expected. What do I do?
Our Genome-wide predesigned siRNAs provide three candidates per target gene. In order to request more than four candidates, order via Custom siRNA request. The siRNA sequences can be verified only after the order has been submitted. If you would like to compare the sequences with publications or to modify your order, please email us at sirna-support@bioneer.co.kr or call us at +82-42-930-8777.

4. Do phosphate groups present on the 5′ or 3′ ends of the synthesized siRNA?
Unless explicitly stated, the 5′ and 3′ ends are capped with - OH groups. Therefore, to order 5′ phosphate-capped siRNAs, you must request for 5′ phosphorylation modification

5. How do I store my siRNAs and how long can I keep them?
siRNAs can normally be kept stable at -20°C for over 1 year. The lyophilized form is especially stable and has a longer shelf-life. Although dissolved siRNAs are stable, contamination of the reconstitution solution with RNase will degrade the product. Also, repeated freeze-thaw cycles accelerate the degradation process. Therefore, we recommend that after you receive the siRNA stock, you reconstitute it and make several aliquots to avoid such freeze-thawing. Because the phosphodiester bonds of the RNA can be broken under high pH conditions, we ask you to take caution, and recommend reconstituting in ultrapure water provided.

6. How do I store my fluorescent dye modified siRNA?
Photobleaching may occur if the fluorescent dye modified siRNA is exposed to light for prolonged periods of time. Therefore, we recommend that you store such siRNAs in a dark container, and store that container in a dark place

7. Can I know how many ng the synthesized product is?
Normally, we will fulfill an order with a guaranteed nmole amount, and the synthesis report will also report the final amount in nmole. If you must have the ng amount to calculate for an experiment, you can convert from nmole to ng by using the formula below. We make it easy for you by giving you the molecular weight of the siRNA sequence in the report. Molecular Weight (g) X mole (nmole) = Mass of siRNA (ng).

8. This is my first siRNA experiment. How do I set my experimental conditions?
One of the most important factors in a siRNA experiment is the assessment of whether the siRNA gets delivered into the cell. Bioneer offers positive controls that can easily indicate whether the siRNA is being delivered successfully.

9. What are some precautions for a siRNA experiment?
Firstly, because not all siRNAs will knock-down the target gene with identical efficiency, you should try 2-3 different sequences to find the best siRNA. Secondly, to make sure that the knock-down affects downstream protein expression miRNA levels should also be measured. Thirdly, verify the knock-down phenotype by using another siRNA designed for the same target gene and show that the same phenotype appears.

10. How do I use 10 nmole of siRNA to transfect cells at 100 nM?
This is a source of confusion for many people. In order to transfect a single well with 100 nM siRNA where the transfection volume is 1 ml, you need 100 pmole of siRNA. 2 µl of 50 µM (50 pmole/µl) stock siRNA solution in 1 ml will yield 100 pmole. If you were to order 10 nmole of a siRNA, it will be sufficient to transfect 100 wells at 100 nM (100 pmole/ml).

11. How do I verify my siRNA transfection efficiency?
You can easily verify the transfection efficiency by transfecting your cells with NC-FITC and observing the cells with a fluorescence microscope. The NC-FITC can also be used as a test reagent to optimize the transfection concentrations of both the siRNA and the transfection reagent.

12. How do I verify the siRNA knockdown efficiency?
The siRNA knockdown efficiency can be verified through various techniques including qPCR, Northern blot, Western blot etc.

Dilution Protocol

1. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the siRNA pellet is located at the bottom of the tube.
2. Dissolve siRNAs to a convenient stock concentration using the recommended volume of DEPC-D.W. (or RNase-free water) shown in Table 1.
3. Pipette the solution up and down 3-5 times (or vortex briefly).
4. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the solution is collected at the bottom of the tube.
5. Aliquot the siRNAs into small volumes and store at -20˚C. siRNA is stable for 1 year under the specified storage condition. For best results, limit freeze-thaw events for each tube no more than five.

Table 1. Recommended siRNA resuspension volumes and concentrations.

siRNA Amount (nmol)	DEPC-D.W. volume (µl) for desired final concentration	
	100 µM stock	20 µM stock
10	100	500
20	200	1000
50	500	Exceeds tube
100	1000	volume

Transfection Protocol

* We use the Lipofectamine™ RNAiMAX (Invitrogen; Cat. no. 13778) and HeLa cell for transfection procedure.
* This protocol is fixed at 6-well plate *in vitro* culture condition (if you want to change this condition, you have to consider the relative surface area (Table 2) and Invitrogen protocol, when you are seeding the cells into the culture dish).

1. One day (24 hr) before transfection, plate 3.0×10^5 HeLa cells in each well with 2.5 ml of growth medium without antibiotics such that they will be 50-60% confluent at the time of transfection.
2. Remove the growth medium from the 6-well plate before transfection. And add the 500 µl fresh growth medium without serum in each well.
3. For each well to be transfected, prepare siRNA duplex-Lipofectamine™ RNAiMAX complexes as follows.

3-1. Dilute siRNA duplex (making final concentration as 5 nM~100 nM) in 250 µl growth medium (or Opti-MEM® I Reduced Serum medium) without serum. Mix gently by vortex.

3-2. Mix Lipofectamine™ RNAiMAX gently before use, then dilute 3.5 µl in 250 µl medium (or Opti-MEM® I Reduced Serum medium) without serum. Incubate this solution 5 min at room temperature.

3-3. Combine the diluted siRNA duplex with the diluted Lipofectamine™ RNAiMAX. Mix and incubate for 20 min at room temperature.

4. Add the mixture to each well containing HeLa cells, which result 1 ml as total volume. Mix gently by hand rocking the plate back and forth.
5. Incubate the cells for 5-6 hr at 37˚C in CO_2 incubator.
6. Change the medium with fresh one containing serum and incubate the cells 24-48 hr until you are ready to assay for gene knockdown.

Table 2. The relative surface area of *in vitro* cell culture dish and culture media volume

Culture Vessel	Relative surface area	Volume of plating medium
96-well	0.2	100 µl
48-well	0.4	200 µl
24-well	1	500 µl
6-well	5	2.5 ml
60 mm	10	5 ml
100 mm	30	10 ml

02

miRNA Synthesis Service

Overview

A mature form of microRNA (miRNA) consists of 21-25 nucleotide (nt) long, single-stranded RNA molecules that serve as a post-transcriptional regulator of gene expression in eukaryotes. The human genome may encode over 1000 miRNAs, which bind with imperfect complementarity to their target mRNAs, generally within the 3'UTR (untranslated region), and repress protein production by destabilizing the mRNA as well as translational suppression. miRNA-mediated translational plays an important role in wide range of biological process, including development, cell proliferation and differentiation, apoptosis and metabolism.

The biogenesis of miRNAs consists of two sequential processing events. Primary miRNA transcripts (pri-miRNAs), which contain one or multiple stem-loop hairpin structures, are mostly derived from Pol II-mediated transcription. In the first step towards the canonical miRNA maturation pathway, pri-miRNA is cleaved by the microprocessor complex, RNase III enzyme Drosha, to yield the pre-miRNA, a hairpin-shaped intermediate precursor ~70 nt in length. Pre-miRNAs are then exported from the nucleus to the cytoplasm by Exprotin5 protein, where another RNase III enzyme Dicer catalyzes the second processing event for miRNA biogenesis and liberates the mature miRNA duplexes. The mature miRNA duplexes consist of the mature miRNA strand and the miRNA* strand, which are derived from two separate arms of the hairpin stem within the miRNA precursor. The miRNA is loaded into an Argonaute-containing RNA-induced silencing complex (RISC), whereas the miRNA* strand is typically degraded. The Ago:miRNA complex then dissociates from RISC loading complex, and become the core of the RISC complex to regulate post-transcriptional gene repression of specific target miRNAs (Figure 1)

miRNA has provided new insights in biotechnology. Although they were discovered and recognized relatively and recently, miRNAs have been recognized as the most important gene regulators at the post-transcriptional level, and several studies indicated that miRNAs regulate the expression of more than 30% protein coding genes. The accumulating knowledge about their biogenesis, gene expression regulation mechanism and functions will add a new dimension to our understanding about the complex gene regulatory networks. Recent investigations demonstrate that miRNAs have a unique expression profiles in different cancer types at different stages and play an important role in many disease and viral infections. These results suggest that miRNAs can function as a novel biomarker for disease diagnosis and perform a new strategy for miRNA gene therapy.

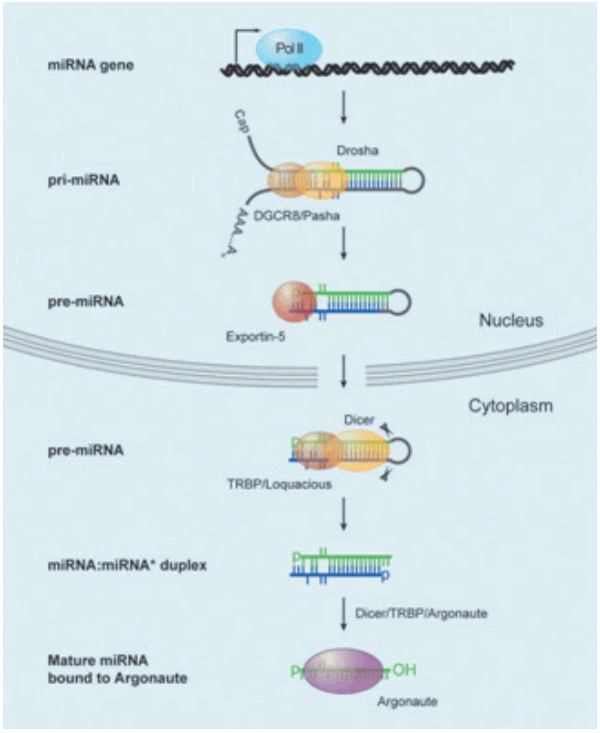


Figure 1. MicroRNA biogenesis (Annu. Rev. Cell Dev. Biol. 2007. 23:175-205)

Description

Bioneer's *AccuTarget*™ miRNA mimics are chemically synthesized, double-stranded RNA oligonucleotides and available for 2,582 Human Mature microRNAs in the miRBase Sequence Database (version 21). *AccuTarget*™ miRNA inhibitors are the single-stranded synthetic inhibitor targeting all human miRNAs in the miRBase Sequence Database. These miRNA mimics & inhibitors are available at 5, 10 and 20 nmole guaranteed yield. We also offer miRNA mimics and inhibitors library sets consisting of predesigned mimics or inhibitors at various small scales (0.25, 0.5, 1, or 2 nmole) in a 96-well plate layout to meet the unique needs of individual customers. In addition, flexible miRNA library sets for customer- specified mimics & inhibitors are also available for the minimum order of 48 ea.

Features and Benefits

Ready-to-transfect miRNA mimics behave like endogenous miRNAs and inhibitors suppress target miRNA activity to study loss-of-function effects after transfection into cells.

- Purification:

  For your more demanding applications, Bioneer's automated HPLC and Bio-RP purification methods ensure high quality, high-throughput miRNA mimics and inhibitors at an affordable price.

- Affordable pricing:

  Bioneer provides a variety of high quality miRNA products at an affordable price.

- Synthesis and QC:

  Bioneer miRNA mimics and inhibitors are produced in clean room facility by fully automated high-throughput miRNA production system. Bioneer miRNA products are assessed by MALDI-TOF Mass spectrometry analysis. Mass spec data is provided with every miRNA mimic and inhibitor. Additionally, miRNA mimics are tested by gel electrophoresis to verify that both RNA strands annealed properly.

All Bioneer miRNA inhibitors are provided as single-stranded miRNA (antisense strand of target miRNA) and all Bioneer miRNA mimics are provided as double-stranded miRNA. Each sense miRNA and an antisense miRNA are QC'ed by MALDI-TOF analysis. Every annealed double-stranded miRNA is then QC-tested using PAGE to confirm proper annealing.

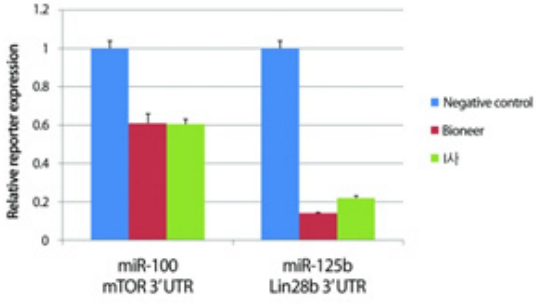


Figure 2. Comparison of reporter gene expression of *AccuTarget*™ microRNA mimics and other company's product.

One day before transfection, 96 well-plate HEK cells in each well with 100 µl of growth medium so that they will be 50-60% confluent at the time of transfection. HEK cells were co-transfected with 10 ng of luciferase vectors (3' UTR reporter and control luciferase) and microRNA using lipofectamine 2000 transfection reagent at a final concentration of 100 nM. Next day, cells were harvested and assayed for luciferase activity. mTOR and Lin28b are validated target mRNA for miR-100 and miR-125b.

Ordering Information

AccuTarget™ Custom miRNAs

Cat. no.	Description	Purification	Guaranteed Yield
SMM-001	*AccuTarget*™ miRNA mimic	Bio-RP	5 nmole
SMM-002	*AccuTarget*™ miRNA mimic	Bio-RP	10 nmole
SMM-003	*AccuTarget*™ miRNA mimic	Bio-RP	20 nmole
SMI-001	*AccuTarget*™ miRNA inhibitor	Bio-RP	5 nmole
SMI-002	*AccuTarget*™ miRNA inhibitor	Bio-RP	10 nmole
SMI-003	*AccuTarget*™ miRNA inhibitor	Bio-RP	20 nmole

AccuTarget™ Library miRNAs

Cat. no.	Description	Purification	Guaranteed Yield
SML-1001	*AccuTarget*™ miRNA mimic	Bio-RP	0.25 nmole
SML-1002	*AccuTarget*™ miRNA mimic	Bio-RP	0.5 nmole
SML-1003	*AccuTarget*™ miRNA mimic	Bio-RP	1 nmole
SML-1004	*AccuTarget*™ miRNA mimic	Bio-RP	2 nmole
SML-2001	*AccuTarget*™ miRNA inhibitor	Bio-RP	0.25 nmole
SML-2002	*AccuTarget*™ miRNA inhibitor	Bio-RP	0.5 nmole
SML-2003	*AccuTarget*™ miRNA inhibitor	Bio-RP	1 nmole
SML-2004	*AccuTarget*™ miRNA inhibitor	Bio-RP	2 nmole

Description

We offer *AccuTarget*™ miRNA mimic controls to optimize assay conditions for miRNA mimic function studies. Both positive and negative controls are provided for miRNA gain-of-function studies using Bioneer's *AccuTarget*™ miRNA mimics.

AccuTarget™ miRNA housekeeping Positive controls target the 3' UTR of the standard housekeeping gene 'GAPDH' and Bioneer's miRNA mimic Negative controls' sequences are based on common miRNA structure for use as negative experimental controls in human, mouse, and rat cells. The negative controls have been analyzed by BLAST against all human, mouse and rat genomic sequences and miRNA sequences in the current miRBase Database. Bioneer offers two universal negative controls for mimics. In addition, *AccuTarget*™ miRNA control Sets consisting of a Positive and two Negative miRNA controls are also available for user convenience.

Features and Benefits

- Excellent performance:

  miRNA Houskeeping Positive controls targeting GAPDH with clear read-out of mimic function (knockdown efficiency of >90%) miRNA mimic Negative controls with minimal sequence identity with miRNAs in human, mouse and rat.
- Purification:

  Fluorescence-labeled Negative controls for conveniently monitoring cellular uptake and/or transfection efficiency
- Affordable pricing:

  Great value for your research dollar

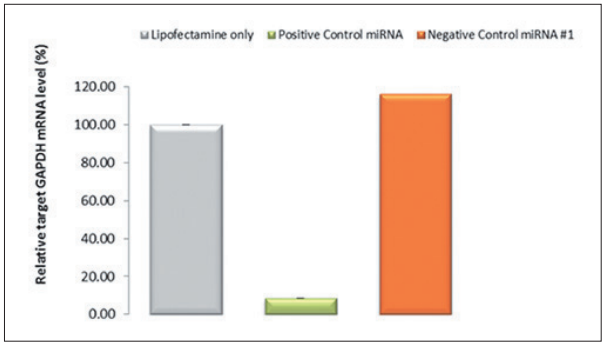


Figure 3. Performances of miRNA mimic Positive and Negative controls.

AccuTarget™ miRNA mimic Positive & Negative Controls were transfected at 20 nM using Lipofectamine™ RNAiMAX into HeLa cell lines and assessed for their ability to decrease target mRNA levels. Down-regulation of GAPDH was determined using the Real-Time quantitative RT-PCR at 48 hr post-transfection using Bioneer's *Exicycler*™ 96 qPCR instrument.

Ordering Information

Cat. no.	Description	Purification	Guaranteed Yield
SMC-1001	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	Bio-RP	5 nmole
SMC-1002	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	Bio-RP	10 nmole
SMC-1003	*AccuTarget*™ miRNA Housekeeping Positive control (GAPDH)	Bio-RP	20 nmole
SMC-2001	*AccuTarget*™ miRNA mimic Negative control #1	Bio-RP	5 nmole
SMC-2002	*AccuTarget*™ miRNA mimic Negative control #1	Bio-RP	10 nmole
SMC-2003	*AccuTarget*™ miRNA mimic Negative control #1	Bio-RP	20 nmole
SMC-3001	*AccuTarget*™ miRNA mimic Negative control #2	Bio-RP	5 nmole
SMC-3002	*AccuTarget*™ miRNA mimic Negative control #2	Bio-RP	10 nmole
SMC-3003	*AccuTarget*™ miRNA mimic Negative control #2	Bio-RP	20 nmole
SMC-4001	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	HPLC	5 nmole
SMC-4002	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	HPLC	10 nmole
SMC-4003	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #1	HPLC	20 nmole
SMC-5001	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	HPLC	5 nmole
SMC-5002	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	HPLC	10 nmole
SMC-5003	*AccuTarget*™ Fluorescein-labeled miRNA mimic Negative Control siRNA #2	HPLC	20 nmole
SMC-2101	*AccuTarget*™ miRNA inhibitor Negative control #1	Bio-RP	5 nmole
SMC-2102	*AccuTarget*™ miRNA inhibitor Negative control #1	Bio-RP	10 nmole
SMC-2103	*AccuTarget*™ miRNA inhibitor Negative control #1	Bio-RP	20 nmole
SMC-3101	*AccuTarget*™ miRNA inhibitor Negative control #2	Bio-RP	5 nmole
SMC-3102	*AccuTarget*™ miRNA inhibitor Negative control #2	Bio-RP	10 nmole
SMC-3103	*AccuTarget*™ miRNA inhibitor Negative control #2	Bio-RP	20 nmole
SMC-4101	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #1	HPLC	5 nmole
SMC-4102	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #1	HPLC	10 nmole
SMC-4103	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #1	HPLC	20 nmole
SMC-5101	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #2	HPLC	5 nmole
SMC-5102	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #2	HPLC	10 nmole
SMC-5103	*AccuTarget*™ Fluorescein-labeled miRNA inhibitor Negative Control siRNA #2	HPLC	20 nmole

03

In vivo siRNA Custom Service: SAMiRNA™

In vivo siRNA Custom Service Using SAMiRNA™ Technology

Overview

SAMiRNA™ (Self-Assembled-Micelle-inhibitory-RNA) custom synthesis service provides a customized option to provide a target specific delivery of siRNA in interest in vivo, essential for target validation and therapeutics development. We offer a complete solution for RNAi (gene silencing) studies from siRNA, miRNA, in vivo si-/miRNA delivery using SAMiRNA™ to all the components and services required for quantitative analyses of targeted genes, using a novel SAMiRNA™ nanoparticle technology.

Description

Since the inception, Bioneer Corporation has been currently developing SAMiRNA™ drugs for the clinical applications through partnership with pharmaceutical companies. To foster open innovation and new partnership opportunities in various therapeutic areas, we are offering valuable opportunities to utilize this second-generation siRNA technology at an affordable price for translational in vivo siRNA studies. This also includes research and technical support teams ensure the top-quality products and services to meet the unique needs of clients from companies and research institutions worldwide. Our world-class teams have highly profiled expertise in nucleic acid manufacturing and it's *in vivo* applications

For that this service provides a seamless approach to place an order for custom SAMiRNA™ of interest, based on a target gene of interest. Upon manufacturing, the final product is then delivered, which can simply be re-suspended and used for the efficacy testing in animals. Upon customer's interest, we also provide additional screening in vitro and validation services that include specific siRNA design, in vitro validation of siRNA for further optimization studies and assess knockdown efficiency in vivo. All of our SAMiRNA™ service and products are provided for Research Use Only. For further development, please contact us for the licensing program.

SAMiRNA™ (Self-Assembled-Micelle-inhibitory-RNA)

One major hurdles in RNA interference (RNAi)-based therapeutics is the proper delivery of siRNA in interest to target tissue and its adverse side effects caused by the specific type of a siRNA delivery vehicle (Table 1). To mediate this, Bioneer has developed SAMiRNA™ (Self-Assembled-Micelle-inhibitory-RNA), which is a novel single-molecular synthetic siRNA, conjugated with lipid and hydrophilic polymer, spontaneously assembled as a stable nanoparticle (NP) with protective PEG coat and lipid core in the nano-scale size level. This system is optimized for systemic delivery and localization in either vascularized tumors through Enhanced Permeability and Retention (EPR) effect with no other reagents need and formulation process. Such novel approach of synthesis provides solutions for the development of RNAi-based drugs. As a second-generation RNAi drug technology, SAMiRNA™ is synthesized as a single chemical entity as a form of nanoparticle (NP), manufactured by one-step automated solid phase synthesis requiring no formulation process and no innate immune response.

The therapeutic potential of SAMiRNA™ is highlighted with negligible toxicity, outstanding for its *in vivo* serum stability, as well as its target gene silencing efficacy in various animal disease models including cancer and lung fibrosis models. In addition, SAMiRNA™ has been shown to induce no innate immune response as demonstrated for a variety of diseases in human PBMC test and high dose-administrated rodent models. This is due to Bioneer's extensive experience in both nucleic acid chemistry and large scale nucleic acid manufacturing capacity and further advancing into clinical development pipelines with top-quality DNA/RNA oligos and siRNA worldwide for 20 years.

Service Details

- Custom designed siRNA synthesis

  Custom siRNA oligos are offered based on the sequences of your interest. Up to 30-mer siRNA including a choice of 32 different 3' overhangs can be synthesized with a variety of modification options for expanded specificity. With this, SAMiRNA™ consists of proprietary polymer -conjugated siRNA that is provided purified, annealed, and lyophilized which can simply be resuspended for efficacy tests in animals

- Genome-wide predesigned mouse, rat and human siRNA

  Genome-wide predesigned siRNA library is available for mouse, rat or human. Bioneer's proprietary Turbo-si designer algorithm designs siRNA from your gene of interest that provides superb knock-down efficiencies of specific targets by binding to the structural hindrance-free region and avoiding off target effect.

- SAMiRNA™ nanoparticle synthesis for *in vivo* siRNA delivery and efficacy/biodistribution tests using animal models

  siRNA contains high lipo-phillic cell membrane barrier and is known to get rapidly degraded when delivered into the blood stream *in vivo*, triggering undesirable immune responses. Thus, SAMiRNA™ resolves all of the limitations, from studies conducted in solid tumor model.

  The siRNA oligos can be manufactured in the form of SAMiRNA™ with SAMiRNA™ nanoparticles (NP) can be provided upon request.

  Upon manufacturing, the reagent can then be re-suspended in PBS and injected these into animals.

- *In vitro* screen/validation of siRNAs of choice lead optimization service

  To achieve and validate siRNA with highest knockdown efficiency, Bioneer recommends "*in vitro* screening and validation service", instead of choosing from in silico. This approach is based on constructing and transfecting up to 100 siRNAs into cells individually, aiming specific mRNAs of interest. Its results in the knockdown efficiencies are ranked and analyzed by RT-qPCR. Upon selection, we determine candidates of most potent siRNAs and SAMiRNA™ nanoparticles (NP) by revalidating *in vitro* before shipment.

- Quantitation of functional knockdown of target gene by Real-Time RT-qPCR (*AccuPower*® qPCR Array Service)

  Bioneer also offers qPCR Array service for quantitation of functional knockdown of target gene by real-time RT-qPCR. The process follows the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines. Experts conduct the experiments and provide data in a short period of time. For more information, please visit "qPCR Array Service". Bioneer's SAMiRNA™ Services provides a rapid on-line quotation at siRNA@bioneer.com for siRNA; qPCRarray@bioneer.com for *AccuPower*® qPCR array service. Please let us know the details of your project, so that we can provide you with a quotation and a timeline estimate

Ordering Information

Bioneer's SAMiRNA™ Custom Services is provided through a SAMiRNA™ ordering system with comprehensive service packages. Please let us know the details of your project, so that we can provide you with an accurate quotation and timeline estimate.

* The formation of SAMiRNA™ nano-particle will be guaranteed upon re-suspension by providing QC data regarding the size and PDI information of the nanoparticle.

SAMiRNA™	Order scale of SAMiRNA™ (nmole)	Number of mouse (intravenous injections)	Price ($)
Custom Designed SAMiRNA™	100	5 mg/kg -> 5 mouse 1 mg/kg -> 25 mouse	Inquire
	500	5 mg/kg -> 25 mouse 1 mg/kg -> 125 mouse	
	1,000	5 mg/kg -> 50 mouse 1 mg/kg -> 250 mouse	
Control SAMiRNA™	100	5 mg/kg -> 5 mouse 1 mg/kg -> 25 mouse	Inquire
	500	5 mg/kg -> 25 mouse 1 mg/kg -> 125 mouse	
	1,000	5 mg/kg -> 50 mouse 1 mg/kg -> 250 mouse	
Fluorescence conjugated SAMiRNA™ (FITC, Cy5.5 etc)	100	5 mg/kg -> 5 mouse 1 mg/kg -> 25 mouse	Inquire

Description

Best-in-class RNAi prodrug technology: SAMiRNA™ is the most unique and effective RNAi prodrug developed to date for the treatment of cancer and other diseases.

- Best-in-class RNAi drug SAMiRNA™ overcomes the Unmet needs in siRNA drug development.

Challenges in siRNA delivery	SAMiRNA™ system
Rapid clearance and degradation in serum	Improved serum stability
Toxicity of delivery systems	No detectable liver toxicity or innate immune response
Limited tissue specificity	Tumor tissue targeting capabilities
Low silencing potency	Long lasting in vivo silencing efficiency

- A new class of single molecule-based self-assembling Nanoparticles

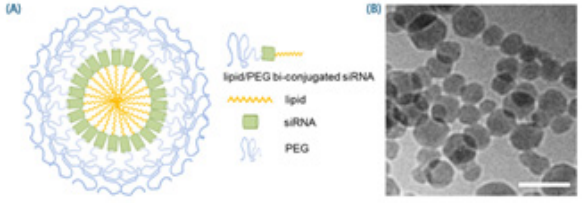


Figure 1. Structure of SAMiRNA™ Nanoparticle.

(A) Schematic diagram of SAMiRNA™ (B) Cryo-TEM images of SAM-siRNA Nanoparticles (scale bar = 100 nm).

Pre-clinical Data for SAMiRNA™

- siRNA Prodrug: highly stable in circulation with siRNA release and activity only after metabolism within target cells
- *In vivo* efficacy validated in animal disease models: completion of preclinical tests for cancer treatment (Figure 2)
- Cancer cell-specific delivery (Figure 3)
- Outstanding serum stability (Figure 4)
- Extremely low toxicity and cytokine induction (Figure 5-7)
- Combined with various targeting moieties, SAMiRNA™ nanoparticles can target various organs of interest, for example, liver

1. Long-lasting *in vivo* efficacy validated in mouse cancer models

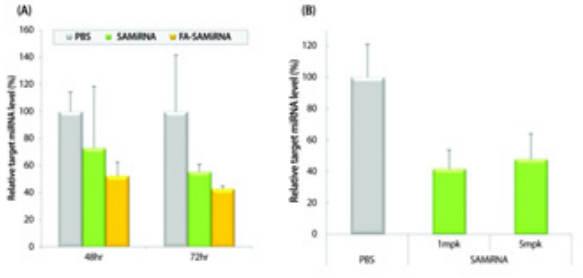


Figure 2. In vivo silencing of target mRNA by SAMiRNA™ Nanoparticles.

(A) Tumor bearing mice were intravenously injected with saline (PBS), or with a single 5 mg/kg dose of either survivin-SAMiRNA, or tumor-targeting folic acid (FA)-conjugated survivin-SAMiRNA. Mice were sacrificed at denoted time points and survivin mRNA levels in isolated tumor cell masses were subsequently measured using Real-Time PCR. (B) Tumor bearing mice were intravenously injected with saline (PBS) and survivin-SAMiRNA Nanoparticle at a single 1 or 5 mg/kg dose. Mice were sacrificed at 72 hr post injection and survivin mRNA levels in isolated tumor cell mass were subsequently measured using Real-Time PCR.

2. Cancer cell-specific delivery

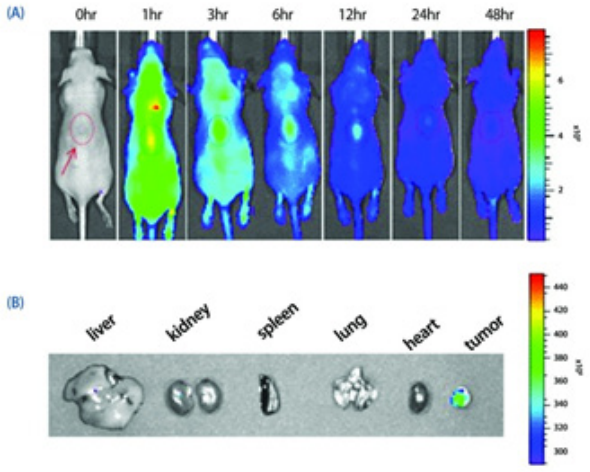


Figure 3. *In vivo* targeting of SAMiRNA™ Nanoparticles to s.c. grafted tumor.

(A) Time-dependent *in vivo* tumor targeting specificity of Cy5.5-labeled SAMiRNA, delivered via i.v. to tumor-bearing nude mice. (B) Images of various organs extracted from treated mice 12 hr after injection. No fluorescence was detectable outside of the tumor proper.

3. *In vivo* PK/PD Quantification of i.v. administrated SAMiRNA™

Shows significantly enhanced stability compared to modified siRNA (2'-O-Methyl siRNA).

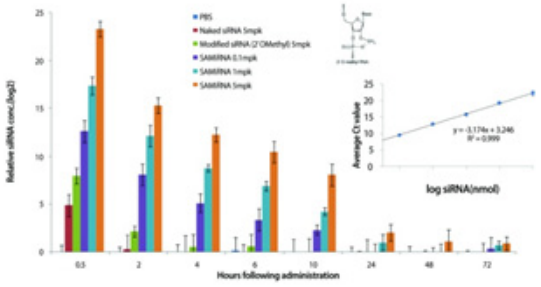


Figure 4. Quantification of siRNA in plasma from mice i.v. injected with SAMiRNA™.

Mouse blood was extracted at indicated time points (hr post-injection) and SAMiRNA™ and siRNA levels in isolated plasma were measured by qRT-PCR assay. The linear regression of the amplification curve shows an excellent R2 value for this assay.

4. No detectable adverse effects

*Mouse

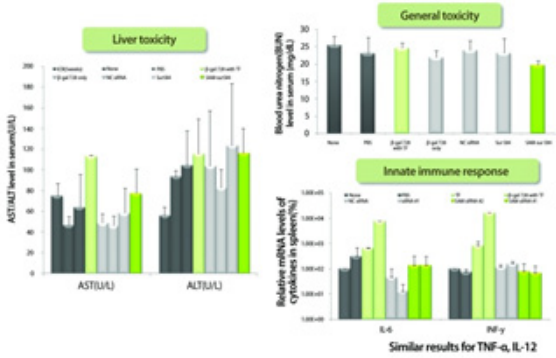


Figure 5. General toxicity test of SAMiRNA™.

Naked siRNA and SAMiRNA™ were i.v. injected into ICR mice in order to evaluate toxicity. Serum was collected 6 or 24 hr post-injection and toxicity markers were analyzed. None (not-injected), ICR 5 weeks (average value from normal 5-week old ICR mice, from published literature), b-gal 728 (beta-galactosidase siRNA), b-gal 728 with TF (beta-galactosidase siRNA mixed with *in vivo* MegaFectine from QBiogene), NC siRNA (negative control siRNA), Sur584 (survivin siRNA), SAM-sur584 (survivin siRNA-containing SAMiRNA).

*Human blood cell

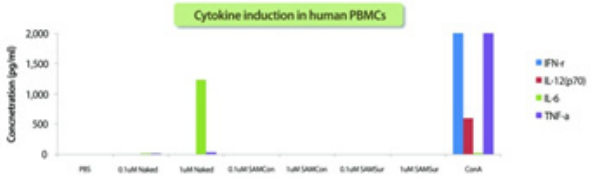


Figure 6. Cytokine response to SAMiRNA™ in human PBMC.

Human whole blood was incubated with 0.1 or 1 μM of naked siRNA or SAMiRNA™ for 24 hr at 37°C and the resulting supernatant was measured for various cytokine induction. Concanavalin A (ConA) was used as positive control

*Toxicity test of SAMiRNA-Survivin including 28-day repeated dose

1) General Toxicology Study

(a) Acute toxicity

- No acute toxicity including bodyweight changes and histological abnormalities were observed

Five aminals (male and female each) pre-group were treated with 100 mg/kg dose i.v. (positive group) or 0 mg/kg (placebo control group), then their fatal rate, symptom, weight change as well as autopsy were evaluated.

(1) No fatality was observed during evaluation period

(2) No symptom related with treatment of testing material was observed.

(3) No change related with treatment of testing material was observed in weight measurement.

(4) Autopsy shows no unusual result was observed related with treatment of testing material.

(b) Repeated dose 28-Day

- No clinically significant or dose-dependent changes were observed post-treatment in hematology, chemistry, urinalysis, coagulation parameters, reticulocyte counts, complement levels.

(1) No fatality was observed during evaluation period.

(2) No symptoms related with treatment of testing material were observed in both male and female treatment groups.

(3) No changes related with treatment of testing material were observed in weight measurement in both male and female treatment groups.

(4) No changes related with treatment of testing material were observed in feeding amount in both male and female treatment groups.

(5) No changes related with treatment of testing material were observed in eye examination in both male and female treatment groups.

(6) Numbers of platelet were decreased in female, 100 mg/kg/day group in hematological analysis.

(7) No changes related with treatment of testing material were observed in blood biochemical evaluation in both male and female treatment groups

(8) No changes related with treatment of testing material were observed in urine evaluation in both male and female treatment groups.

(9) No significant effects related with treatment of testing material were observed in histopathological examination in both male- and female treatment groups.

- NOAEL (No Observed Adverse Effect Level) of SAMiRNA™ was 100 mg/kg /day.

2) Genetic Toxicology Study

(a) Ames test

- No significant induction for mutagenic activity was observed with highest dose tested, 5,000 ㎍ per plate

(b) Chromosome aberration assay

- No significant aberrations in chromosomes were noticed with highest dose tested, 5,000 ㎍ per plate.

5. Safety Assessment

Safety evaluation through 28-day repeated dose and genetic tox studies demonstrated that SAMiRNA™ is an exceptionally safe and efficient drug candidate.

Figure 7. *In vivo* SAMiRNA™ blood chemical and hematological analysis.

SAMiRNA™ was i.v. injected into 6-weeks old mouse (ICR) for 3 days, 1 time per day, then toxicity due to that treatment was evaluated.

SAMiRNA™ was treated with 10 mg/kg concentration. At 24 hr after the final treatment whole blood as well as serum were collected for blood chemical analysis and hematological analysis. (1) No changes related with treatment of testing material were observed in blood biochemical analysis. (2) No changes related with treatment of testing material were observed in hematological analysis.