SAMiRNA™ Custom Service

SAMiRNA™ Custom Services
SAMiRNA™ Technical Specs

SAMiRNA™
Phone: +82-42-930-8777 Email: SAMiRNA-support@bioneer.com

Life Science Product

01

In vivo siRNA Delivery Using SAMiRNA™ Custom Service

01 *In vivo* siRNA Delivery Using SAMiRNA™ Custom Service

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 SAMiRNATM 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 its 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.

Features and Benefits

Delivery challenges overcome by SAMiRNA™ technology

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

SAMiRNA™ (Self-Assembled-Micelle-interfering-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

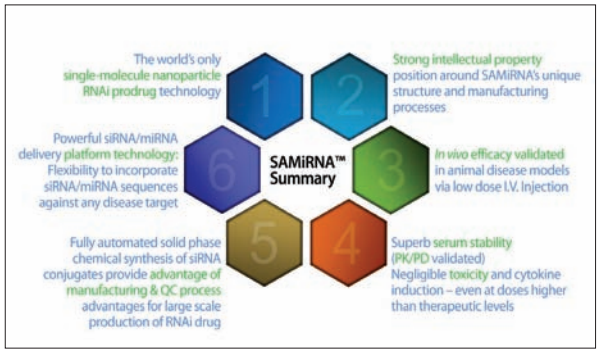
- **Custom SAMiRNA™ nanoparticle synthesis for *in vivo* siRNA delivery, and efficacy/biodistribution tests using animal models**
  siRNA contains high lipo-phillic cell membrane barrier and is known to get rapidly degraded when delivered into the blood stream *in vivo*, triggering undesirable immune 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* screening and validation of siRNAs for lead optimization**
  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 qRT-PCR (*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

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.



Key features of SAMiRNA™

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

Unmet needs in siRNA drug development	SAMiRNA™
Adverse effects	No detectable liver toxicity or innate immune response
Delivery (Issues with other technologies: rapid clearance and degradation in blood, No target tissue specificity, low *in vivo* efficacy)	Outstanding serum stability (PK/PD validated), tumor/tissue targeting capabilities verified, long lasting in vivo efficacy validated in animal disease models
Manufacturing cost	One-step automated solid-phase synthesis of SAMiRNA™ and rapid chromatographic purification process: enormous advantage allowing for economical large scale production of SAMiRNA™
QC processes	Since SAMiRNA™ is a Single Chemical Entity the QC process is dramatically simplified
Expansion of indications	Powerful siRNA delivery platform technology with flexibility to incorporate siRNA (or miRNA or antisense) sequences against any disease target; Your target gene(s) can be easily transformed into SAMiRNA™ nanoparticle!

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

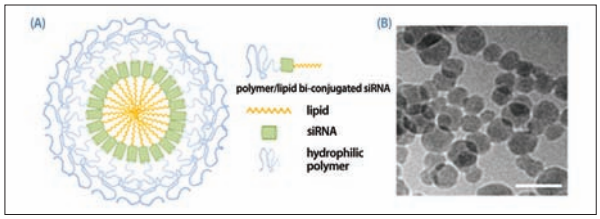


Figure 1. Structure of SAMiRNA™ Nanoparticle.
(A) Schematic diagram of SAMiRNA™ (B) Cryo-TEM images of SAM-siRNA Nanoparticles (scale bar = 100 nm).

Pre-clinical data for SAMiRNA™

- siRNA Prodrug: highly stable in circulation with siRNA release and activity only after metabolism within target cells
- Outstanding serum stability (Figure 4)
- *In vivo* efficacy validated in animal disease models: completion of preclinical tests for cancer treatment (Figure 2)
- 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

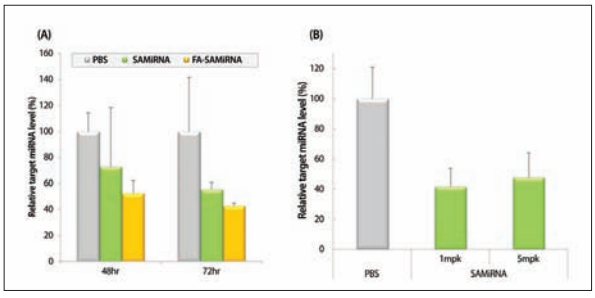


Figure 2. In vivo silencing of target mRNA by SAMiRNA™ Nanoparticels.
(A) Tumor bearing mice were intravenously injected with saline (PBS), or with a single 5 mg/kg dose of either survivin-SAMiRNA, or tumor-targeting folic acid (FA)-conjugated survivin-SAMiRNA. Mice were sacrificed at denoted time points 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 NP 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. Tumor-specific targeting of SAMiRNA™ in mouse cancer models

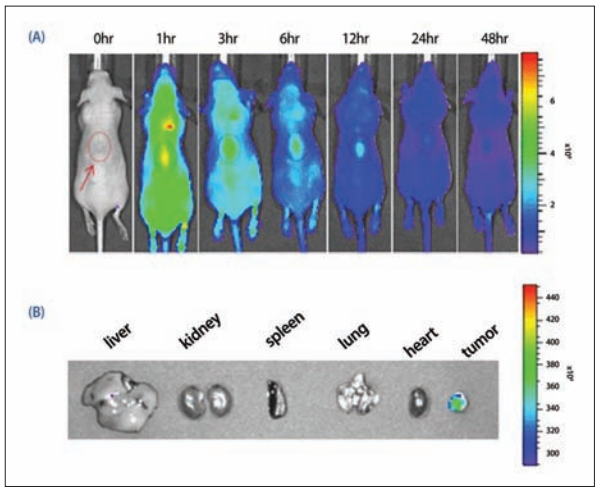


Figure 3. *In vivo* targeting of SAMiRNA™ Nanoparticles to s.c. grafted tumor.
(A) Time-dependent in vivo tumor targeting specificity of Cy5.5-labeled SAMiRNA, delivered via i.v. to tumor-bearing nude mice. (B) Images of various organs extracted from treated mice 12 hr after injection. No fluorescence 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

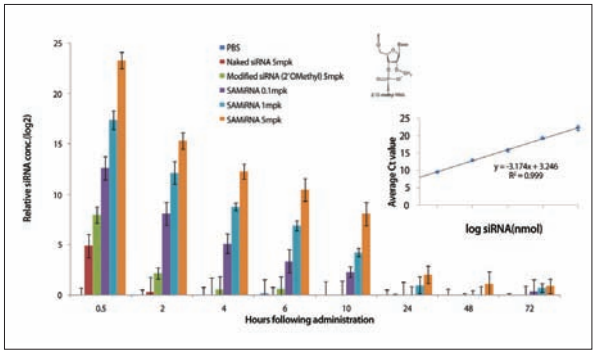


Figure 4. Quantification of siRNA in plasma from mice i.v. injected with SAMiRNA™.
Mouse blood was extracted at indicated time points (hours post-injection) and SAMiRNA™ and siRNA levels in isolated plasma were measured by qRT-PCR assay. The linear regression of the amplification curve shows an excellent R2 value for this assay.

4. No detectable adverse effects

*Mouse

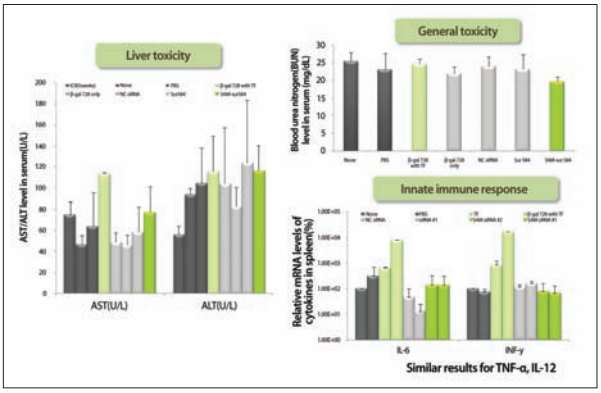


Figure 5. General toxicity test of SAMiRNA™.
Naked siRNA and SAMiRNA™ were i.v. injected into ICR mice in order to evaluate toxicity. Serum was collected 6 or 24 hr post-injection and toxicity markers were analyzed. None (not-injected), ICR 5 weeks (average value from normal 5-week old ICR mice, from 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

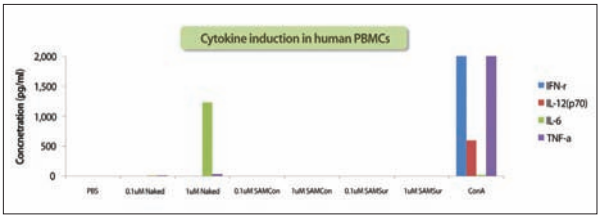


Figure 6. Cytokine response to SAMiRNA™ in human PBMC.
Human whole blood was incubated with 0.1 or 1 μM of naked siRNA or SAMiRNA™ for 24 hr at 37°C and the resulting supernatant was measured for various cytokine induction. Concanavalin A (ConA) was used as positive control.

*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

(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
- 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 μg per plate

(b) Chromosome aberration assay
- No significant aberrations in chromosomes were noticed with highest dose tested, 5,000 μg 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.

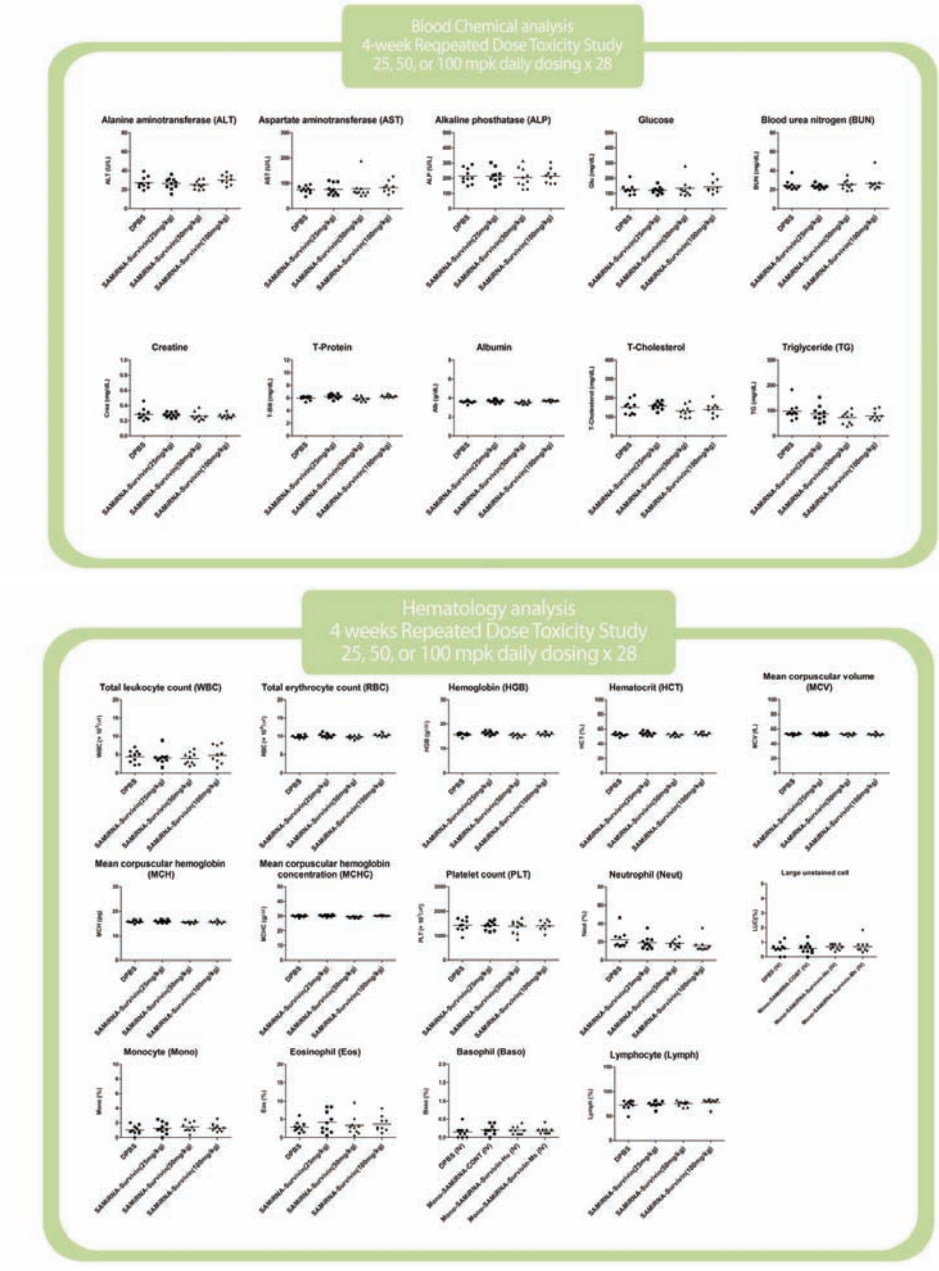


Figure 7. *In vivo* SAMiRNA™ blood chemical and hematological analysis.

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

Order scale of SAMiRNA™ (nmole)	Number of mouse (intravenous injections)	Price
100	5 mg/kg -> 5 mouse 1 mg/kg -> 25 mouse	Inquire
500	5 mg/kg -> 25 mouse 1 mg/kg -> 125 mouse	
1000	5 mg/kg -> 50 mouse 1 mg/kg -> 250 mouse	

- **SAMiRNA™ ordering**

SAMiRNA™ is synthesized by incorporating siRNA sequence(s) into SAMiRNA™ platform. siRNA sequences can be picked from either custom siRNAs or Bioneer's pre-designed siRNA pool.

1. Custom siRNA

SAMiRNA™s loaded with customized siRNAs are available. Please let us know a sequence of your own functional anti-sense strand. SAMiRNA™ nano-particle of duplex annealed with a corresponding complementary sequence will be provided.

2. Pre-designed siRNA

Pre-designed siRNA sequences for SAMiRNA™ synthesis can be searched in the Bioneer AccuTarget Pre-designed siRNA database by Gene ID, Symbol, Synonyms, Description and/or Accession numbers.

3. SAMiRNA™ controls

Varieties of SAMiRNA™ controls are available to confirm the functional activities and/or the delivery of SAMiRNA™ to target tissues. SAMiRNA™s constituted with siRNA sequences aiming at non-target (negative control), GFP and luciferase are provided. In addition, SAMiRNATMs can be labeled with various fluorescent dyes to detect the localization of SAMiRNA™ in vivo animal models.

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