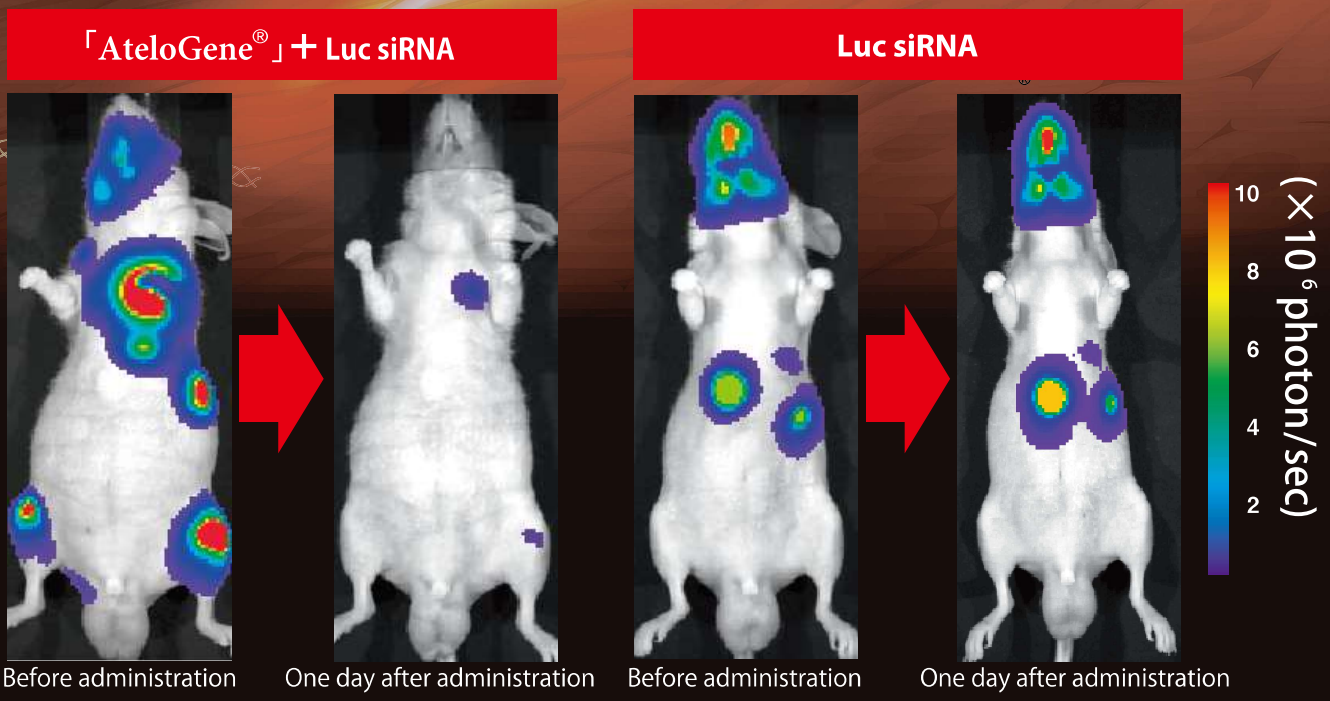
KOKEN

in vivo siRNA / miRNATransfection Kits

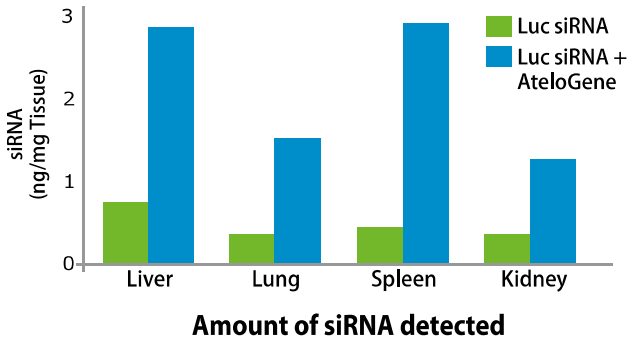
AteloGene®

Local & Systemic Use

Atelcollagen-Based Transfection:
Efficient Delivery / High Biocompatibility / Easy Handling



Efficient siRNA delivery to Luciferase expressing metastatic prostate cancer model.



Amount of siRNA detected

25 µg of siRNA complexed with AteloGene® "Systemic Use" formula or 25 µg naked siRNA was administered to each animal via tail vein injection. Effective systemic delivery of Luc siRNA by in AteloGene®-complexed siRNA was confirmed *in vivo* imaging for Luciferase fluorescence one day after administration.

COSMO BIO CO., LTD.

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in vivo siRNA/miRNA transfection Kits AteloGene® Local & Systemic Use

Outline

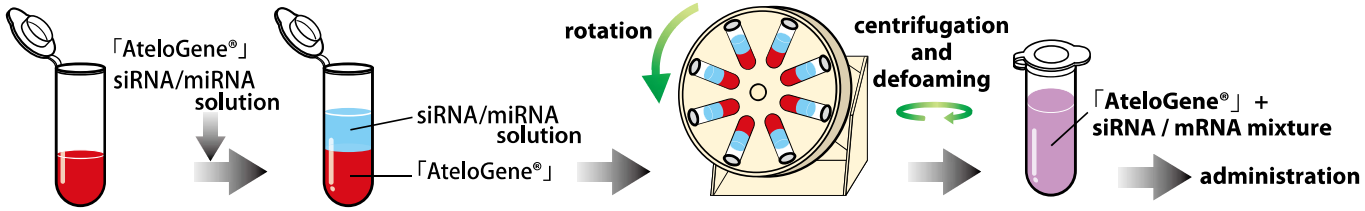
Atelocollagen, the main component of AteloGene®, forms siRNA/miRNA-atelocollagen complexes by mixing with appropriate quantity and ratio of synthetic siRNA/miRNA. Because siRNA-atelocollagen complexes repress the degradation of nucleic acid, it is optimal for *in vivo* transfection, and siRNA/miRNA is effectively delivered and introduced into the cells.

AteloGene® Local Use is designed for localized administration because of its gelation capability. Gelated siRNA/miRNA-atelocollagen complexes remain at the injection site and siRNA/miRNA is delivered into the cells effectively.

AteloGene® Systemic Use is suitable for systemic administration via tail vein injection because it does not gelate, and siRNA/miRNA is delivered effectively via the bloodstream throughout the whole body.

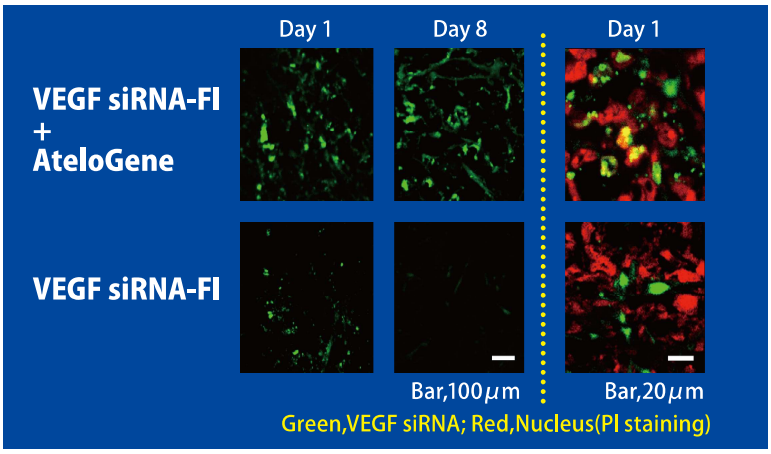
How to use

AteloGene® procedures are simple and easy.
Mix equal volumes of AteloGene® and siRNA/miRNA solution (Local Use: 5-10 μM, Systemic Use: 20-40 μM) and administer the siRNA/miRNA-AteloGene® mixture to the mouse.



Stabilization of siRNA *in vivo* by AteloGene®

Inhibition of tumor proliferation by administration of VEGF siRNA



AteloGene® Local Use formula was mixed with fluorescent labeled vascular endothelial growth factor (VEGF) siRNA and injected into a subcutaneous tumor. Compared to VEGF siRNA alone, siRNA complexed with AteloGene® was delivered to tumor cells effectively and siRNA was still detected after 8 days. Remarkable inhibition of tumor proliferation was also confirmed. (Data source: Dr. Y. Takei, Nagoya University, Japan - See reference #10 on the last page)

AteloGene® injection has little effect on background gene expression.

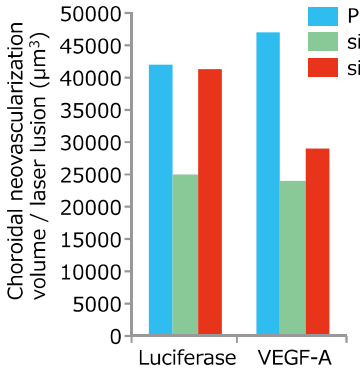
Comparison of hepatotoxicity from microarray results

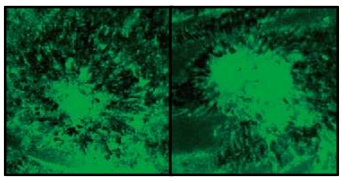
Up-regulated gene ontology category	P-Value < 0.0001	
	AteloGene	Liposome
0009607: response to biotic stimulus	P>0.05	2.37×10^{-64}
0006952: defense response	P>0.05	1.09×10^{-56}
0006955: immune response	0.0375	9.84×10^{-54}
0009613: response to pest, pathogen or parasite	P>0.05	1.15×10^{-28}
0043207: response to external biotic stimulus	P>0.05	6.45×10^{-26}
0009615: response to virus	P>0.05	1.25×10^{-18}
0009605: response to external stimulus	P>0.05	1.71×10^{-17}
0019882: antigen presentation	0.0047	6.59×10^{-16}
0006950: response to stress	P>0.05	6.17×10^{-15}
0006954: inflammatory response	P>0.05	2.30×10^{-10}
0006953: acute-phase response	P>0.05	1.07×10^{-9}
0045087: innate immune response	P>0.05	7.55×10^{-6}
0006917: induction of apoptosis	P>0.05	9.98×10^{-6}
0012502: induction of programmed cell death	P>0.05	9.98×10^{-6}
0043068: positive regulation of programmed cell death	P>0.05	8.33×10^{-5}

The effects of AteloGene® Systemic Use injection versus liposome injection on mouse liver-cell gene expression was compared by microarry analysis.
Expression levels of genes from several ontological categories, including apoptosis-related genes, were upregulated strongly by liposome injection whereas AteloGene® injection showed hardly any effect.
(See reference #6 on the last page)

AteloGene® inhibits the immune responses to ds DNA

Nonspecific angiogenesis suppression due to dsRNA administration



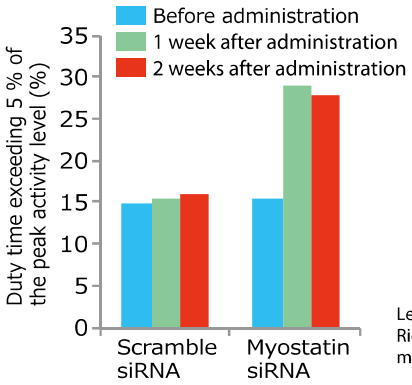


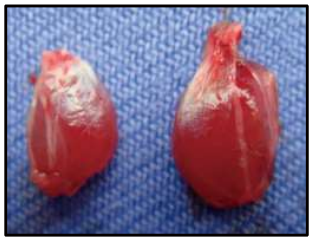
Left : Luc siRNA
Right: Luc siRNA+ AteloGene®
Confocal imaging of the mouse retina pigment epithelium-choroid membrane complex, one week after dosage.

Luc siRNA and VEGF-A siRNA were administrated to vitreous humor of mouse choroidal neovascularization (CNV) model with AteloGene® Systemic Use.
siRNA non-specific angiogenesis was observed only in the siRNA administrated model, while siRNA specific angiogenesis was observed in the siRNA and AteloGene® administrated model.
Moreover, expression of IFN-γ were remarkably increased in the siRNA administrated model. Thus, AteloGene® is suitable for *in vivo* nucleic acid transfection.
(Data source: Dr. M. Nozaki, Nagoya City university, Japan
-See reference #1 on the last page)

AteloGene® is also suitable for nucleic acid transfection into muscle

Increase of muscle quantity and activity by Myostatin-siRNA administration



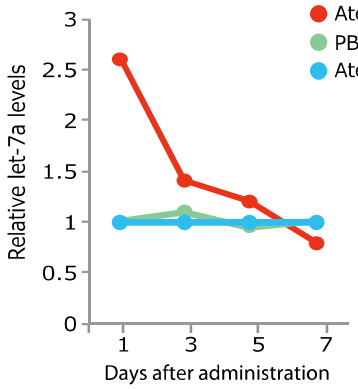


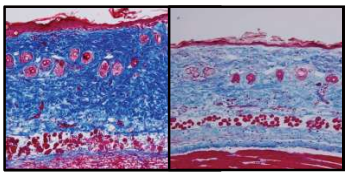
Left : Scramble siRNA
Right : Myostatin siRNA administrated mouse masseter (2 weeks after administration)

Myostatin siRNA was administrated to masseter of a muscular dystrophy model mouse by AteloGene® Local Use. Compared to the control, muscle mass and muscle fiber remarkably increased in mouse administrated Myostatin siRNA and AteloGene®.
Moreover, activity of muscle measured by an electromyogram showed significant increase compared to the control group. This study also showed high efficiency of nucleic acid transfection to muscle using AteloGene®.
(Data source : Dr. E. Tanaka, Tokushima university, Japan
-See reference #2 on the last page)

AteloGene® is effective for nucleic acid transfection into skin

An inhibition experiment : let-7a administration to Bleomycin-induced skin fibrosis model



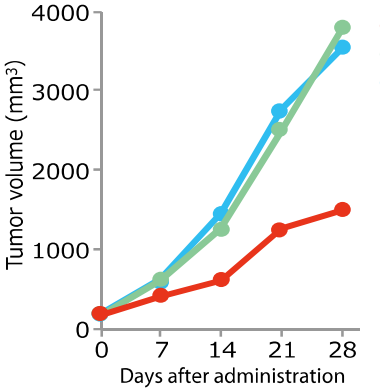


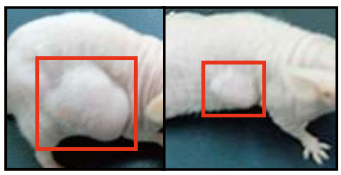
Left : Control , Right : let-7a administrated dorsal skin dyed by Masson's trichrome

Expression of let-7a in Bleomycin-induced scleroderma model was increased after intraperitoneal administration of let-7a and AteloGene Systemic Use®. Skin hypertrophy and collagen fiber increase were surpressed in the mouse administrated with let-7a and AteloGene®, suggesting that AteloGene® has high nucleic acid introduction efficiency.
(Data source: Dr. K. Makino and Dr. M. Jinnin, Kumamoto University, Japan
-See reference #3 on the last page)

AteloGene® is widely used for Cancer Research

Tumor proliferation reduced by repressing 3 types of small RNA expression of Human RGM249 gene





AteloGene® + 3 siRNA that (Left : Mock, Right : 3 siRNA) administrated to tumor bearing model. (4 weeks after administration)

Three siRNAs corresponding to 3 types of small RNA from Human RGM249 gene were administrated to the subcutaneous tumor model of Human malignant melanoma using AteloGene® Local Use. Remarkably, reduction of tumor proliferation was observed in the siRNA with AteloGene® administrated model.
(Data source: Dr. M. Miura, Tottori University, Japan
-See reference #4 on the last page)

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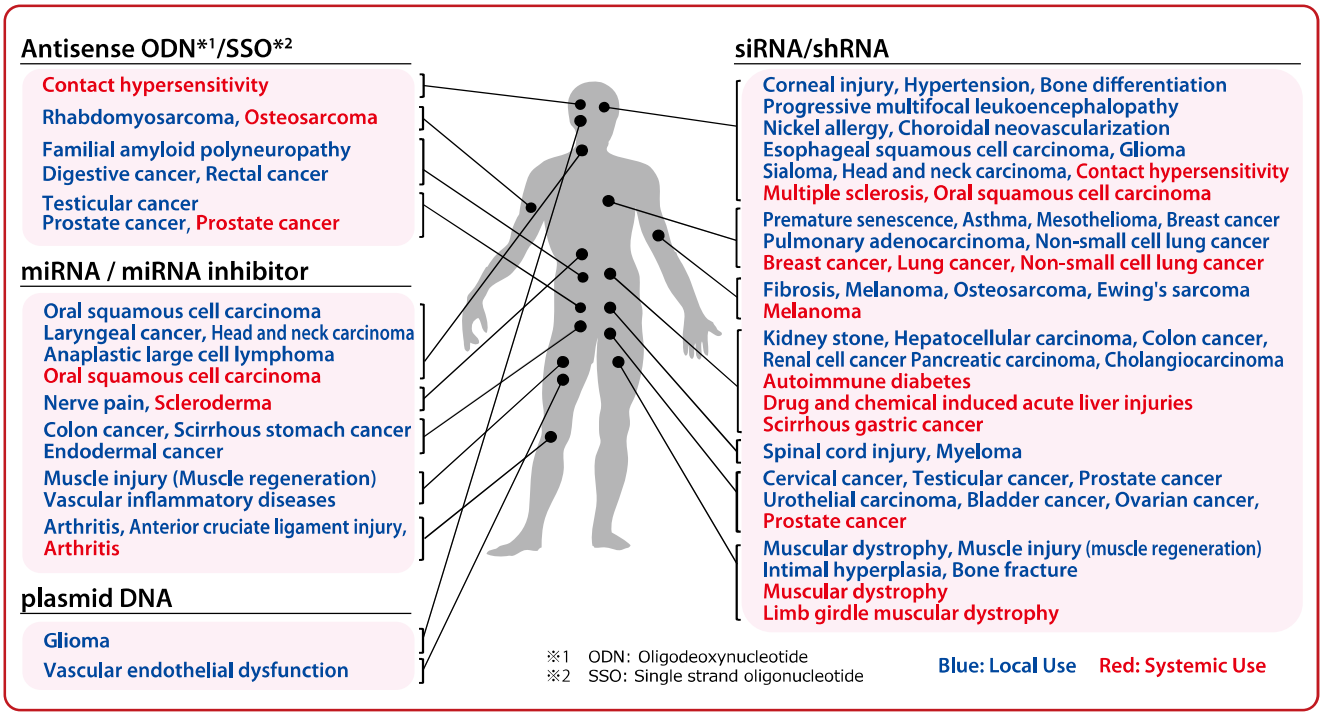
Reported Drug Delivery Systems using atelocollagen



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Ordering information

Description	Cat. No.	Quantity
AteloGene® Local Use	**KOU-1392**	**1 Kit***
AteloGene® Systemic Use	**KOU-1393**	**1 Kit***

* Sufficient for 10 injections.

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