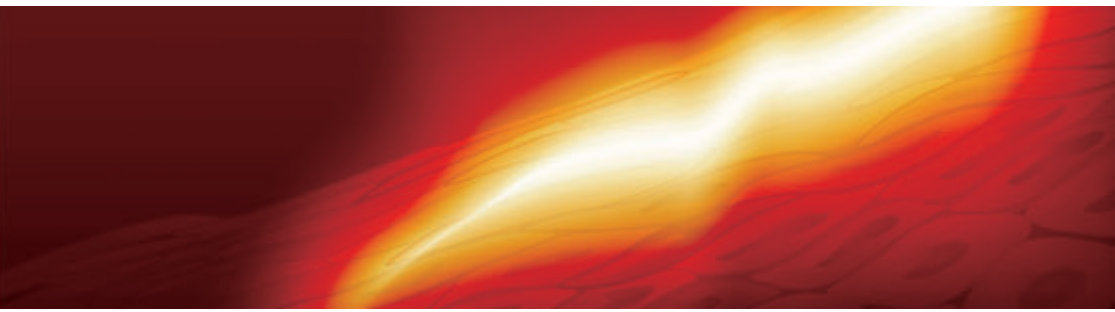
in vivo siRNA/miRNA Transfection Kit

AteloGene®

Systemic Use

Manual



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Contents

Purpose of use

This kit is designed for delivering siRNA/miRNA into animal tissues, particularly mouse, to introduce siRNA/miRNA into cells via systemic administrations.

Characteristics

- Prolonged RNAi effect because atelocollagen, the main component of "AteloGene®", forms complexes with siRNA/miRNA [1].
- Since "AteloGene®" is made from atelocollagen, it is nontoxic [2].
- "AteloGene® Systemic Use" does not form a gel and siRNA/miRNA are efficiently delivered throughout the entire body via the blood circulation following tail vein injection [3].

Principle

Atelocollagen is positively charged and can thus electrostatically form complexes with nucleic acids when they are mixed together at appropriate concentrations and ratios. The complexes protect nucleic acids from being degraded by nucleases.

Kit contents

This kit is intended for 10 administrations at the recommended dose.

① Prefilled syringe (filled with "AteloGene®")	600 μL × 2 syringes
600 μL is sufficient for 5 administrations, including losses during the preparation steps.	
② 10×siRNA buffer (also applicable to miRNA)	3 mL × 1 bottle
③ Sterilized water	3 mL × 1 bottle
④ Microtube	2.0 mL × 2 tubes
⑤ Disposable syringe	1 mL × 2 syringes
⑥ 18G needle (for ejection and suction)	4 needles
⑦ 26G needle (for injection)	2 needles

Devices and reagents required other than those in the kit

- siRNA/miRNA (PAGE- or HPLC- purified grade, such as "AteloSiLence® " in vivo grade siRNA/miRNA, is recommended.)
- Container for preparation of siRNA/miRNA solution (sterilized, RNase free)
- Cooling device (crushed ice, cold block, etc.)
- Tube rotator (that can tumble and agitate such as TAITEC RT-5, Bibby scientific SB3, etc.)
- Pipetter and tips (sterilized, RNase free)
- High-speed refrigerated centrifuge
- Anesthetic (as required)
- Mouse holder (as required)
- Cotton swab immersed in ethanol

Storage

Storage temperature: 2-10℃ (do not freeze)

Effective period: 3years from the manufacturing date indicated on the box

Precautions for storage

- Fibril formation and thermal denaturation occur in "AteloGene®" at a temperature higher than 20℃. Do not use "AteloGene® " that was once fibrillated or thermally denatured.
- Freezing "AteloGene®" causes bubbles in the mixture and the dispersion of components. Do not use "AteloGene®" that was once frozen.
- ② 10×siRNA buffer may develop crystals during storage. In this case, heat approximately 37℃ to completely dissolve the crystals prior to use.

Precautions and disclaimer

1) **Do not use "AteloGene® " for any purpose other than research use. Application to the human body is strictly prohibited.**

2) Be sure to read the instruction manual before use. The manufactur is not liable for the results of usage by methods other than that described in the instruction manual.

The expected effects may not always be obtained depending on the siRNA/miRNA sequences, administration targets or methods.

Preparation instructions

In this section, the numbers ①-⑦ refer to the "Kit contents" on page 2.
Implement measures to secure an Rnase-free environment,
as far as possible, to avoid the degradation of siRNA/miRNA in advance.

1) Preparation of "AteloGene® "

Attach the ⑥18G needle to the ①"AteloGene® " prefilled syringe. Eject the whole amount (600 μL) into the ④Microtube. After ejection, cool the microtube containing "AteloGene®" on ice.

> Note) An excess amount of "AteloGene® " is provided in the ①Prefilled syringe so that 600 μL of "AteloGene®" can be injected into the ④Microtube regardless of losses during the preparation steps.

2) Preparation of siRNA/miRNA solution

Prepare 20-40 μM siRNA/miRNA solutions for systemic administrations.

a. Using siRNA/miRNA stock solutions
Adjust the concentration of the siRNA/miRNA solution with ②10×siRNA buffer and ③Sterilized water to a buffer concentration of 1×. Cool on ice.

b. Using lyophilized siRNA/miRNA
Prepare 1×siRNA buffer by diluting ②10×siRNA buffer with ③Sterilized water.
Add 1×siRNA buffer to prepare siRNA/miRNA solutions of the desired concentrations.
Cool on ice.

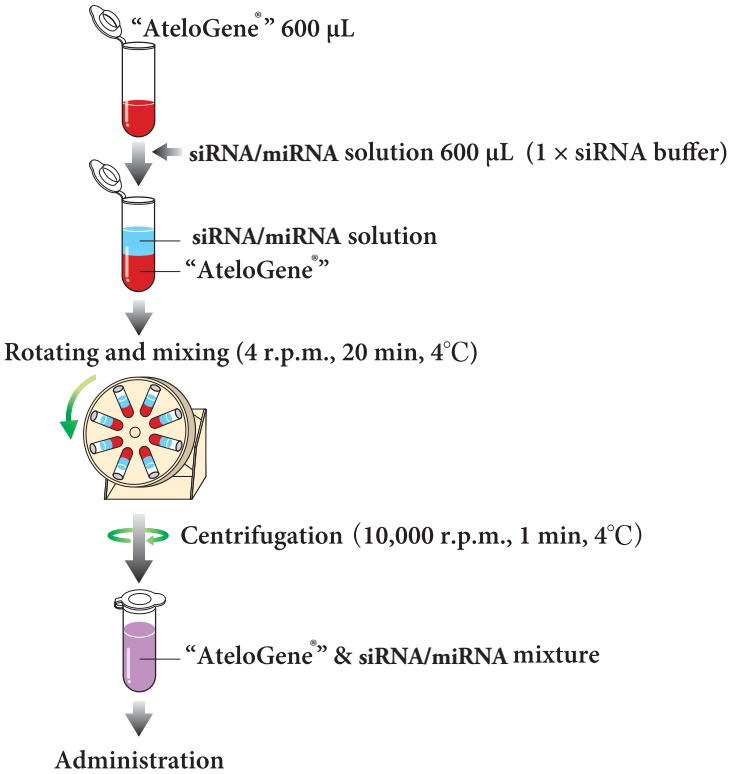
> Note) Although other buffers such as TE buffer may be used for preparing the siRNA/miRNA solution, ②10×siRNA buffer is recommended for obtaining the best results.

3) Preparation of "AteloGene® " & siRNA/miRNA complex

While cooling on ice, gently pour 600 µL of the siRNA/miRNA solution onto 600 µL of "AteloGene®" in the ④Microtube. Slowly rotate and mix the solutions at 4℃ for 20 minutes. To avoid forming bubbles, the rotation speed should be approximately 4 r.p.m. (in the case of a rotator with a diameter of 20 cm).

4) Deformation of bubbles and preparation for administration

Centrifuge the mixture at 10,000 r.p.m. for 1 minute at 4℃ to deform bubbles. Attach the ⑥18G needle to the ⑤Disposable syringe and slowly draw the mixture while avoiding forming bubbles. Replace the needle with the ⑦26G needle and keep the syringe refrigerated until immediately before administration.



Method for systemic administration

The standard single dose for a mouse is 200 μL of "AteloGene®"&siRNA/miRNA mixture
The upper limit of the one-time dose is 200 μL. Be sure not to exceed this dose.

Repeated administrations, e.g. once a week for 3 weeks, twice a week for 2 weeks, etc., are also applicable.

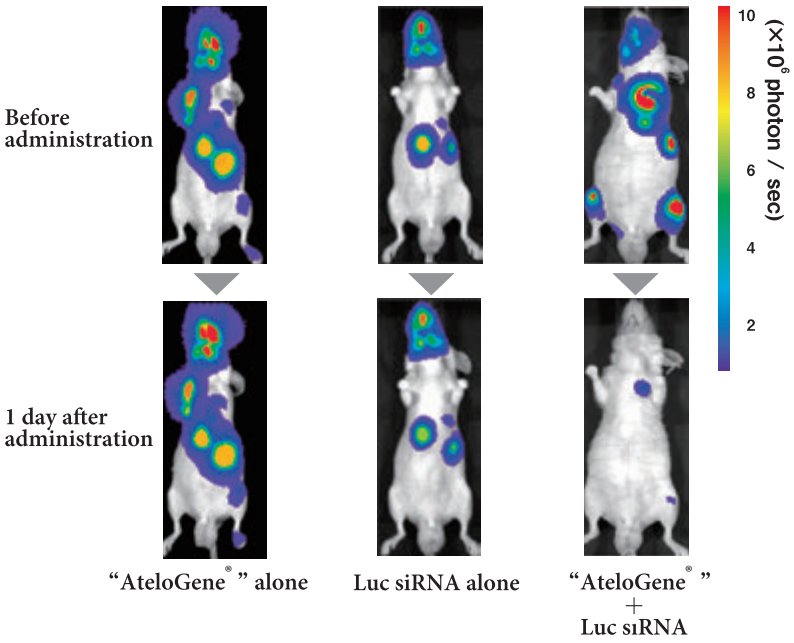
1) **Restrain the mouse, if necessary.**
2) **Wipe the tail of the mouse to disinfect it and to increase the visibility of the vein.**
3) **Insert the needle into the tail vein at a position approximately 1/4 to 1/3 of the tail length from the tail end.**
4) **Confirm that the needle has entered the vessel and then slowly inject 200 μL of the mixture. The injection speed should be less than 10 μL/s.**

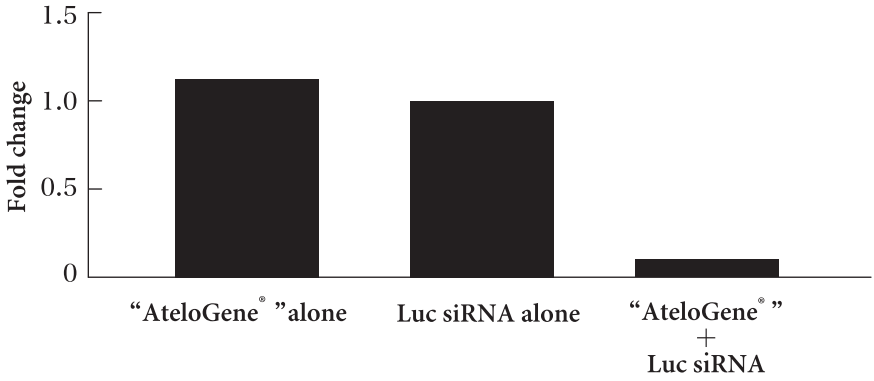
Evaluation of the siRNA/miRNA transfection effect

Effects of siRNA/miRNA transfections with "AteloGene®" may differ depending on the siRNA/miRNA sequences, expression levels of target genes, target tissues, etc. Please consider optimizing siRNA/miRNA concentrations, administration frequencies, and timings for administrations and evaluations accordingly.

【Experimental example】

Suppression of gene expression **in a metastatic tumor mouse model.**





After systemic administration of a luciferase siRNA (Luc siRNA) & "AteloGene®" mixture to metastatic tumors expressing luciferase, luciferase expression was markedly reduced when compared to the control.

References

1) Minakuchi Y, *et al.* (2004) *Nucleic Acids Res.* 32(13):e109.

2) Ogawa S, *et al.* (2011) *J Toxicol Sci.* 36(6):751-62.

3) Takeshita F, *et al.* (2005) *Proc Natl Acad Sci U S A.* 102(34):12177-82.

Please visit "AteloGene®" web site to see many other references.

atelocollagen.com/atelogene/

Notice

The technique of introducing nucleic acid components using this kit or collagen was developed by the National Cancer Center, Sumitomo Pharma Co., Ltd. and Koken Co., Ltd. (PCT/JP02/06137).

AteloGene®

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