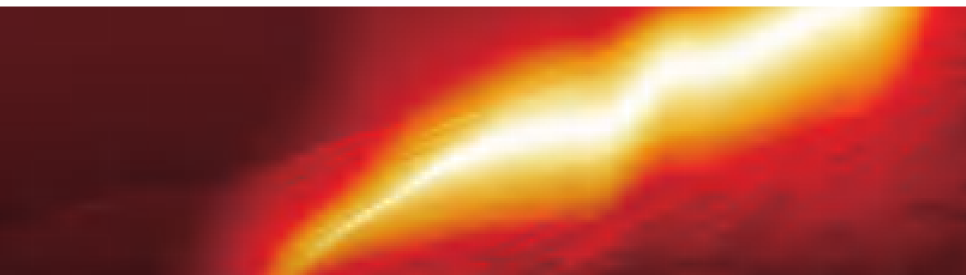
in vivo siRNA/miRNA Transfection Kit

AteloGene® Local Use

"Quick Gelation"

Manual



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Purpose of use

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

Characteristics

- Prolonged RNAi effect because atelocollagen, the main component of AteloGene® products, form complexes with siRNA/miRNA[1].
- Since AteloGene® products are made from atelocollagen, it is nontoxic[2].
- AteloGene® Local Use gelates in the body and siRNA/miRNA are slowly released from the gel at the site of administration[3].
- AteloGene® Local Use "Quick Gelation" ("AteloGene® QG") has been modified to increase transfection efficiency by focusing on faster gel formation at the site of administration.

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 15 administrations at the recommended dose.

① Prefilled syringe (filled with "AteloGene® QG") ——— 540 μL × 3 syringe
540 μL is sufficient for 5 administrations, including losses during the preparation steps.

② QG buffer ——— 1.5 mL × 3 tubes

③ 2 mL microtube ——— 4 tubes
A spare tube is included.

④ 18G needle (for ejection and suction) ——— 8 needles
2 spare needles are included.

Devices and reagents required other than those in the kit

- 1 mL disposable syringe
- 27G needle (for injection)
- siRNA/miRNA (PAGE- or HPLC-purified grade, such as "AteloSiLence®" *in vivo* grade siRNA/miRNA, is recommended.)
- Container and water 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

Storage

Storage temperature: 2-10°C (do not freeze)

Precautions for storage:

- Gelation and thermal denaturation occur in "AteloGene® QG" at a temperature higher than 20°C. Do not use "AteloGene® QG" that was once gelated or thermally denatured.
- Freezing "AteloGene® QG" may cause bubbles and the dispersion of components.

Precautions and disclaimer

1) Do not use "AteloGene® QG" 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 ①-④ refer to the “Kit contents” on page 3. 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® QG”

Attach the ④18G needle to the ①Prefilled syringe. Eject the whole amount (540 µL) into the ③2 mL microtube. After ejection, cool the microtube containing “AteloGene® QG” on ice.

> Note) An excess amount of “AteloGene® QG” is provided in the ①Prefilled syringe so that 540 µL of “AteloGene® QG” can be injected into the ③2.0 mL microtube regardless of losses during the preparation steps.

2) Preparation of siRNA/miRNA solution

Prepare 25 – 50 µM siRNA/miRNA solutions with RNase-free water.

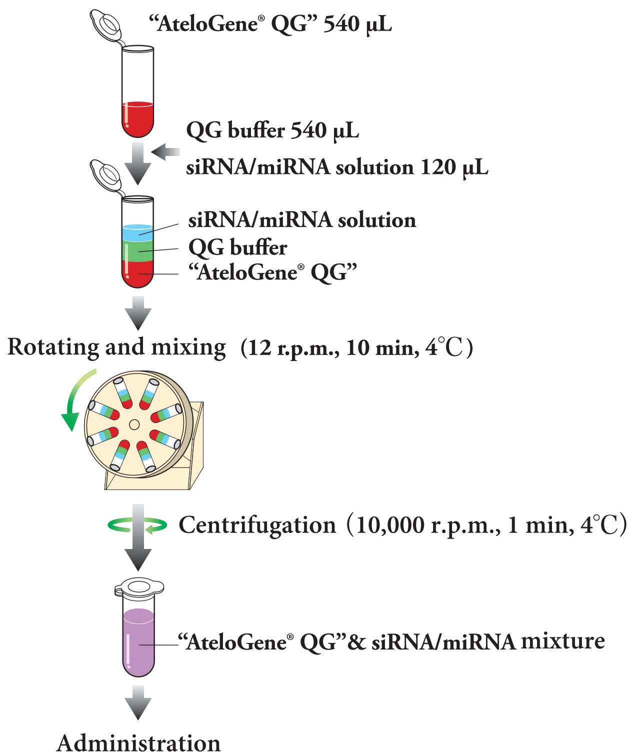
3) Preparation of “AteloGene® QG” & siRNA/miRNA complex

While cooling on ice, gently pour 540 µL of the ②QG buffer and then 120 µL of the siRNA/miRNA solution onto 540 µL of “AteloGene® QG” in the ③2.0 mL microtube. Then,tap the microtube briefly. Rotate and mix the solutions at 4℃ for 10 minutes. To avoid forming bubbles, the rotation speed should be approximately 12 r.p.m. (in the case of a rotator with a diameter of 20 cm).

> Note) Although the mixture may become cloudy during this procedure, it will become a clear solution as the components are thoroughly mixed by rotation.
> Dispose the residual QG buffer and use freshly opened QG buffer each time.

4) Defoamation of bubbles and preparation for administration

Centrifuge the mixture at 10,000 r.p.m. for 1 minute at 4℃ to defoam bubbles. Attach the ④18G needle to the disposable syringe and slowly draw the mixture while avoiding forming bubbles. Replace the needle with the 27G needle and keep the syringe refrigerated until immediately before administration.


"AteloGene® QG" 540 µL
QG buffer 540 µL
siRNA/miRNA solution 120 µL
siRNA/miRNA solution
QG buffer
"AteloGene® QG"
Rotating and mixing (12 r.p.m., 10 min, 4℃)
Centrifugation (10,000 r.p.m., 1 min, 4℃)
"AteloGene® QG"& siRNA/miRNA mixture
Administration

Method for local administration

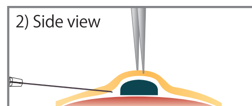
For best results, cover the whole target site with the "AteloGene® QG" & siRNA/miRNA mixture. Although the standard single dose for a mouse is 200 μL of the mixture, adjustments may be required depending on the target sites and sizes. Repeated administrations, e.g., once a week for 3 weeks, twice a week for 2 weeks, etc., are also possible.

Example of local administration

<Administration to subcutaneous tumor>

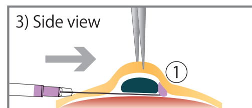
1) Anesthetize the mouse so that it will not be awaken by the puncture.

2) Lift the tumor up with forceps. With the cut surface of the needle tip turned upward, insert the needle to approximately 2-3 mm from the xenografted tumor.

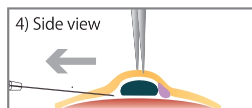


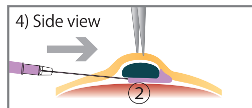
> Tips: It is advised to employ forceps with the needle, which enables you to fine-tune the movement of the needle during the administration processes.

3) Along the bottom of the tumor, advance the needle to the far side of the tumor (①). Inject 1/6-1/8 of the total volume of the "AteloGene® QG" & siRNA/miRNA mixture.



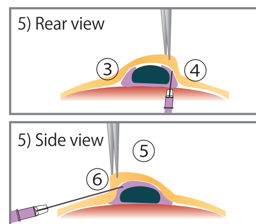
4) Draw the needle towards the puncture point and forward the needle beneath the tumor (②)and inject 1/6-1/8 of the total volume of the mixture.





> Tips: To eject the mixture into target sites, it is necessary to draw the needle close to the puncture point prior to each injection. However avoid pulling the needle out, as it may cause leakage of the mixture.

5) Repeat 4) on both sides (③ and ④), the top (⑤) and the front side (⑥) of the tumor.



Tips: It is advantageous to follow this sequences as the border between the skin and the tumor becomes unclear once the tumor is covered by the mixture.

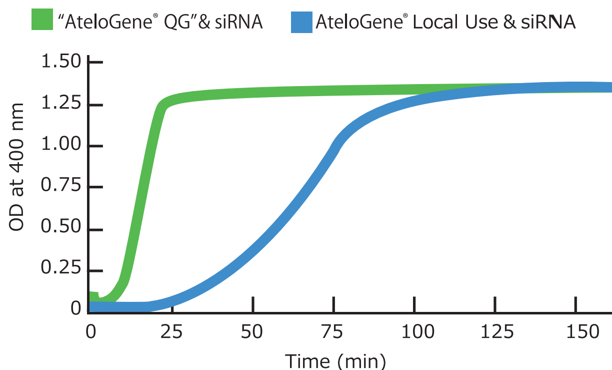
6) If there are any uncovered parts, move the needle around and inject the mixture to cover the tumor evenly.

7) Withdraw the needle gently to avoid leakage of the mixture.

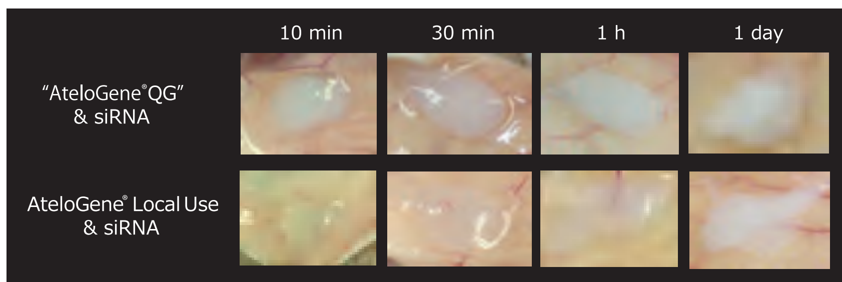
Evaluation of the siRNA/miRNA transfection effect

Effects of siRNA/miRNA transfections with "AteloGene® QG" 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.

Reference data

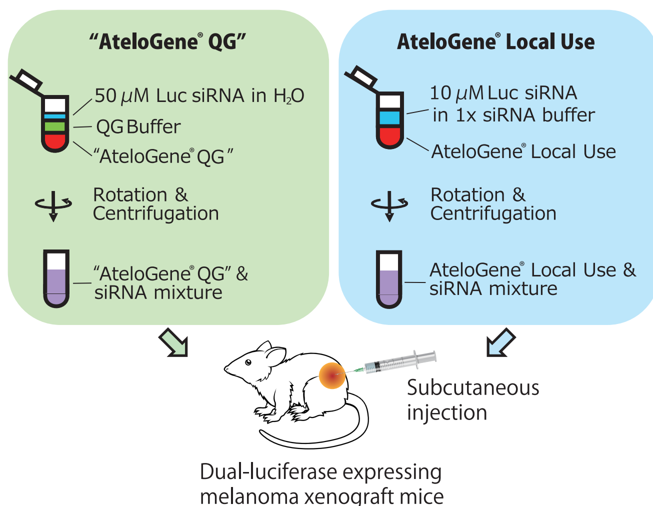


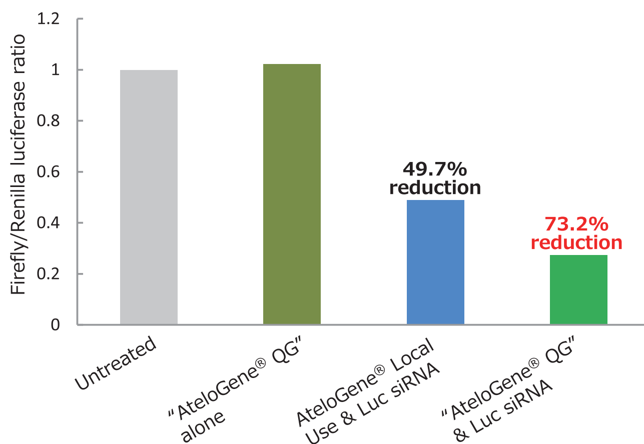
Speed of fibril formation in an "AteloGene® QG" and siRNA mixture (green line) and in an AteloGene® Local Use and siRNA mixture (blue line) at 37°C.



Gel formation by "AteloGene® QG" and siRNA mixture (top panels) and AteloGene® Local Use and siRNA mixture (bottom panels) following subcutaneous injection into mice.

Experimental example





A remarkable reduction of luciferase expression was observed when a luciferase siRNA (Luc siRNA) was co-administered to dual-luciferase expressing melanoma xenograft mice with "AteloGene® QG".

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) Takei Y, *et al.* (2004) *Cancer Res.* 64(10):3365-70.

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

atelocollagen.com/atelogene/

Industrial property rights

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).

The technique of sustained release of pharmaceutical components using this kit or collagen is patented by Koken Co., Ltd. in Japan (JP6186572).

The usage of this product is limited to research purposes and other usages of this product may infringe on the patents.

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