in vivo siRNA Transfection Kit

AteloGene™

Local & Systemic Use

Manual

KOKEN

COSMO BIO CO., LTD.
Inspiration for Life Science
http://www.cosmobio.co.jp/index_e.asp

Contents

Purpose of use

This product is a kit to deliver siRNA into animal tissue by local or systemic administration in mice and then efficiently introducing it into the cells.

Characteristics

- Immediate administration to experimental animals by simple mixing the synthetic siRNA with "AteloGene™" [1-3].
- Efficient *in vivo* transfection of siRNA.
- The effect of RNAi of preventing degradation by RNase persists for a long time.
- "AteloGene™" has no toxicity, and its main component is atelocollagen that demonstrates high biological compatibility.
- It is possible to choose "AteloGene™ Local Use" [1,2] for local administration or "AteloGene™ Systemic Use" [3] for systemic administration by injection to the tail vein.
- Since "AteloGene™ Local Use" for local administration is gelated in the body, siRNA is securely kept at the administration site.
- Since "AteloGene™ Systemic Use" for systemic administration is not gelated in the body but circulated in the blood after injection into the tail vein, siRNA is efficiently delivered to the whole body [3].

Principle

When mixed with synthetic siRNA at an appropriate concentration and ratio, atelocollagen, which is the major component of "AteloGene™" forms a complex appropriate for administration into the body. siRNA that is prepared into a complex with atelocollagen is efficiently delivered *in vivo* and introduced into the cells.

Contents of kit

This kit is intended for 10 times of administration.

①Prefilled syringe (filled with "AteloGene™") Each syringe is for 5 times of administration.	600 μL × 2 syringes
②10 × siRNA buffer	3 mL × 1 bottle
③Sterilized water	3 mL × 1 bottle
④Microtube	2.0 mL × 2 tubes
⑤Disposable syringe	1 mL × 2 syringes
⑥18G needle for ejection and suction	4 needles
⑦26G injection needle	2 needles
⑧Instruction manual	1 leaflet

Devices and reagents required other than the kit

- siRNA(PAGE or HPLC purified grade is recommended. Never fail to use siRNA preserved in water or in desalted and lyophilized condition.)
- Container for preparation of siRNA (sterilized, RNase free)
- Cooling device (crushed ice, cold block, etc.)
- Rotator (that can tumble and agitate. TAITEC RT-5, EYELA MBS-1A, 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: 3 years from the manufacturing date indicated on the box

Precautions for storage

- Gelation and thermal denature occur in "AteloGene™" at a temperature higher than 20°C. Never use "AteloGene™" that was once gelated or thermally denatured.
- Freezing "AteloGene™" causes bubbles in the mixture and the dispersion of components. Never use "AteloGene™" that was once frozen.

Precautions for use

1) This product is a reagent for research use only. Its application to the human body is strictly prohibited. Do not use it for any purpose other than for research purposes.
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. Depending on the siRNA sequence, administration target or administration method, the expected effect may not always be obtained.

Precautions for operation

1) Perform the procedures in a clean environment.
2) Secure a measure to eliminate RNase to avoid the degradation of siRNA by RNase.
3) During storage, ②10×siRNA buffer may generate crystals. In this case, heat it up to about 37°C and completely dissolve it before use.
4) When the ①"AteloGene™" content is squeezed out, ensure that bubbles are not hold.

Procedures

The method of preparing a mixture of "AteloGene™" & siRNA is common to local and systemic administration.

In this regard, ①-⑦ indicated in the following operating procedures refer to the respective "Contents of kit" in the foregoing.

1) Preparation of "AteloGene™"

Set the ⑥18G needle for ejection and suction in the ①"AteloGene™" prefilled syringe. Eject the whole amount (600 μL) into the ④Microtube. After ejection, cool "AteloGene™" transferred into the microtube with ice.

Note) An excessive amount of "AteloGene™" is filled in the ①Prefilled syringe of "AteloGene™". Even if some "AteloGene™" is left in the syringe or the needle for ejection and suction at the time of extrusion, 600 μL of "AteloGene™" is ejected into the ④Microtube.

2) Preparation of siRNA solution

Prepare 5-10 μM and 20-40 μM siRNA solutions for local and systemic administration, respectively. For the preparation of a mixture with "AteloGene™" 600 μL of the above concentration of siRNA solution is necessary. When siRNA is preserved in water at high concentration, use ②10×siRNA buffer and ③Sterilized water to dilute the solution to the above-mentioned siRNA concentration at the final concentration 1×siRNA buffer.

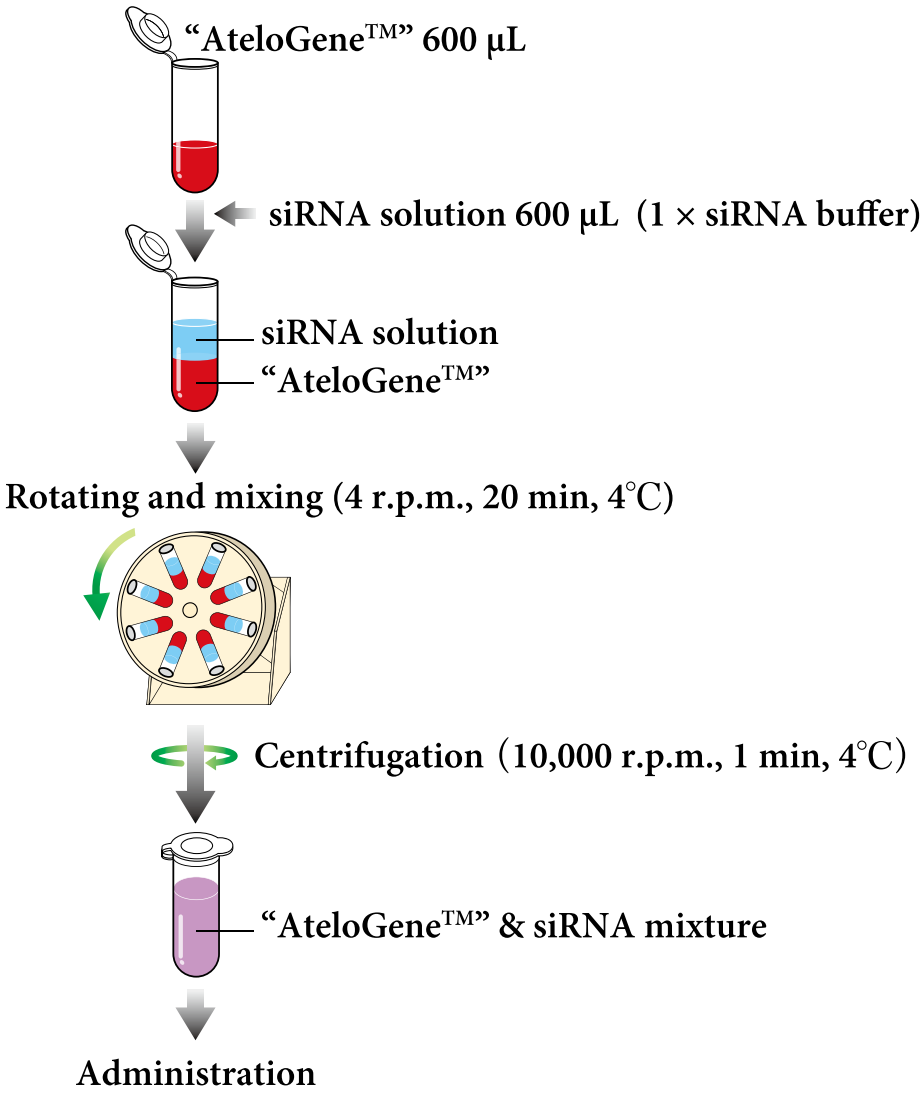
When siRNA is preserved in a lyophilized condition, directly dissolve siRNA with 1×siRNA buffer prepared by diluting ②10×siRNA buffer with ③Sterilized water (②10×siRNA buffer: ③Sterilized water = 1:9) to prepare an siRNA solution of the above-mentioned concentration. Cool the prepared solution with ice. Do not use any buffer other than the buffer attached to the kit. Do not use siRNA that is preserved other than in water or in desalted and lyophilized condition.

3) Preparation of "AteloGene™" & siRNA mixed solution

While cooling with ice, gently pour 600 µL of the siRNA solution (in 2) above) on 600 µL of "AteloGene™" (in 1) above). Using a rotator, slowly rotate and mix the solution at 4°C for 20 minutes and prepare the "AteloGene™" & siRNA mixed solution. The rotating speed is about 4 r.p.m. when a 20-cm-diameter holder is used.

4) Defoaming of mixed solution, preparation for administration

Set the microtube (in 3) above) that contains the "AteloGene™" & siRNA mixed solution in a high-speed refrigerated centrifuge at 4°C. Centrifuge this for 1 minute at 10,000 r.p.m. to defoam the mixed solution. Set the ⑥18G needle for suction in the ⑤Disposable syringe, and slowly draw the mixture while avoiding the incorporation of bubbles. Replace the needle of the syringe with the ⑦26G injection needle and keep the syringe refrigerated until immediately before administration.



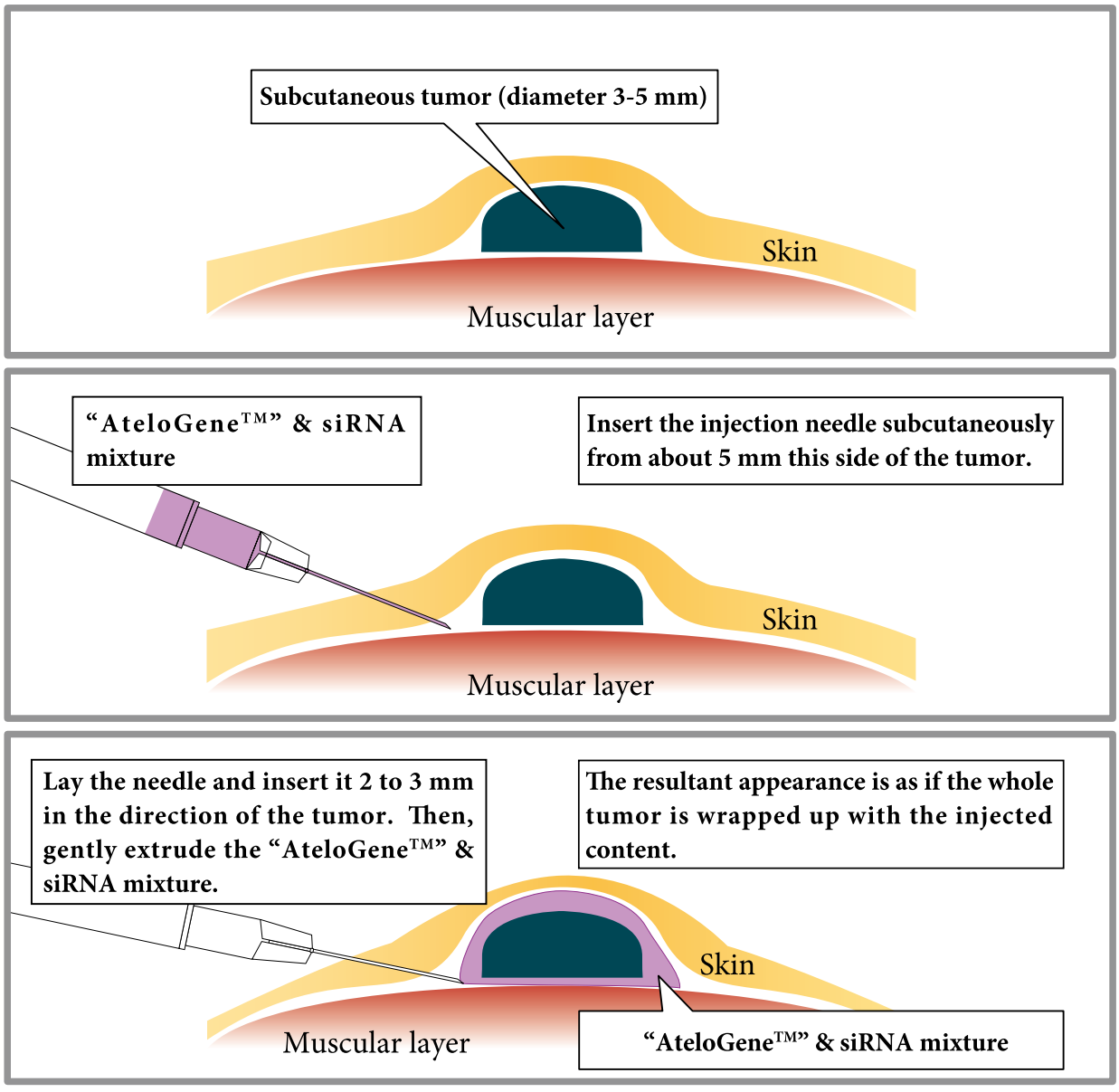
Method of local administration

It is effective to inject the "AteloGene™" & siRNA mixture so as to wrap up the whole target site. The standard single dose for a mouse is 200 μL of "AteloGene™" & siRNA mixture.

Example of local administration

<Administration to subcutaneous tumor>

1) Anesthetize the animal, if necessary.
2) Insert the injection needle from about 5 mm this side of the tumor with the cut face of the needle turned upward.
3) Lay the needle parallel to the skin and insert it for 2 to 3 mm in the direction of the tumor. Then, inject 200 μL of the "AteloGene™" & siRNA mixture gently.



Method of systemic administration

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

1) Anesthetize the animal, if necessary.
2) Fix the mouse.
3) Disinfect the tail of the mouse by wiping it with a cotton swab immersed in ethanol.
4) Insert the needle into the vein at a position 1/4-1/3 from the tail end that is fully inflated.
5) Confirm that the injection needle has entered the vessel and then, slowly inject 200 μL of the "AteloGene™" & siRNA mixture.
6) Confirm the recovery of the mouse.

Confirmation of siRNA Transfection effect

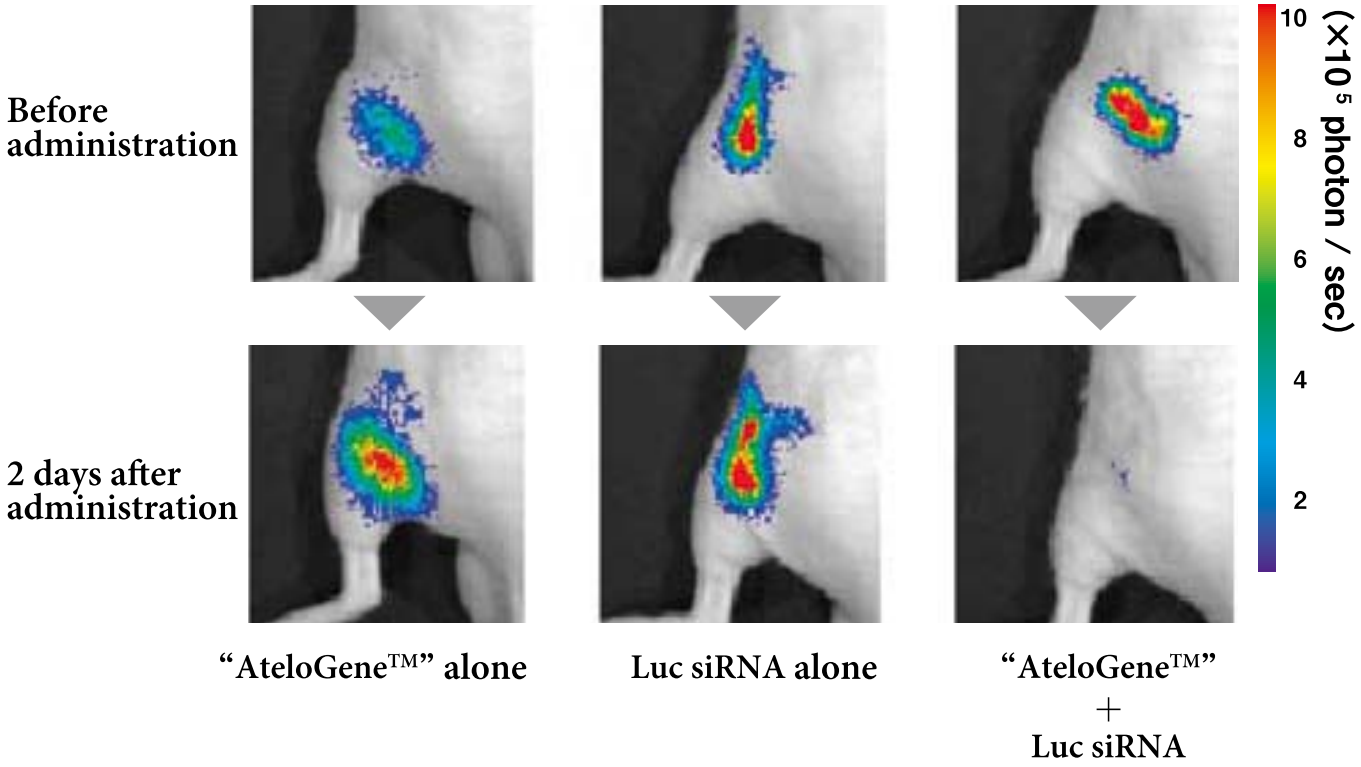
Effect of siRNA transfection by "AteloGene™" differs depending on the siRNA sequence, expression level of the target gene, difference in target tissue, etc. Please investigate the duration required after administration for confirmation of effect and the analysis method.

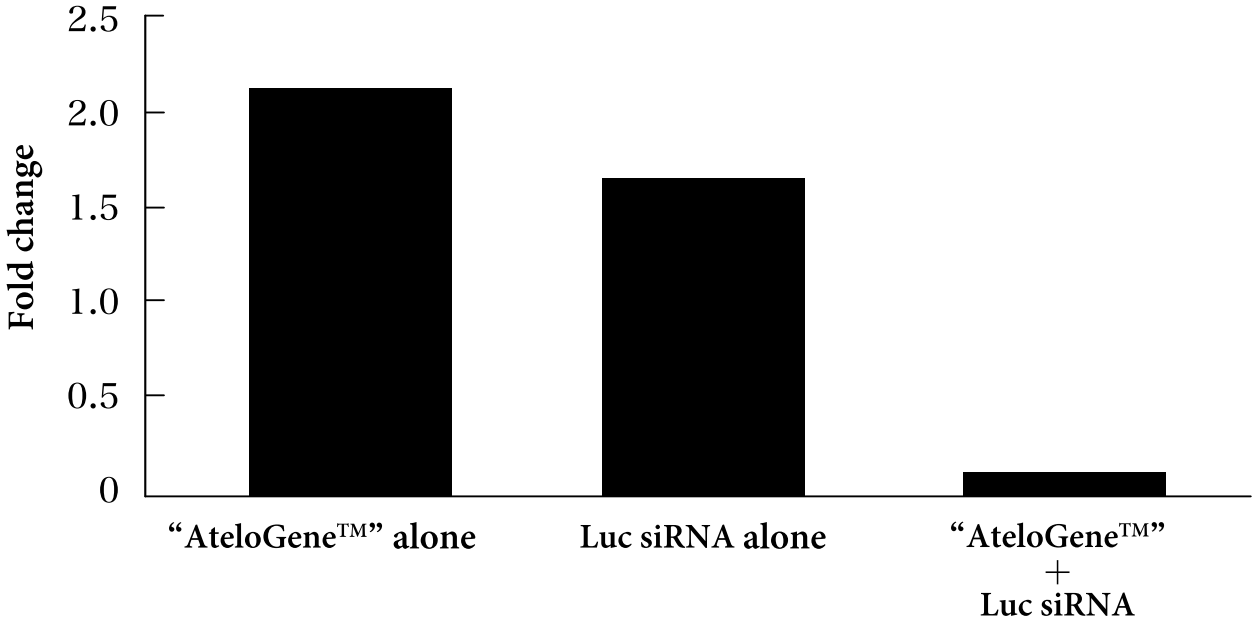
References

1) Y. Takei, *et al., Cancer Res.*, 64, 3365 (2004)
2) Y. Minakuchi, *et al., Nucleic Acids Res.*, 32, e109 (2004)
3) F. Takeshita, *et al., Proc. Nat. Acad. Sci. USA.*, 102, 12177 (2005)

Example
【Local administration】

Suppression of gene expression of subcutaneous tumor[1, 2] in mice.

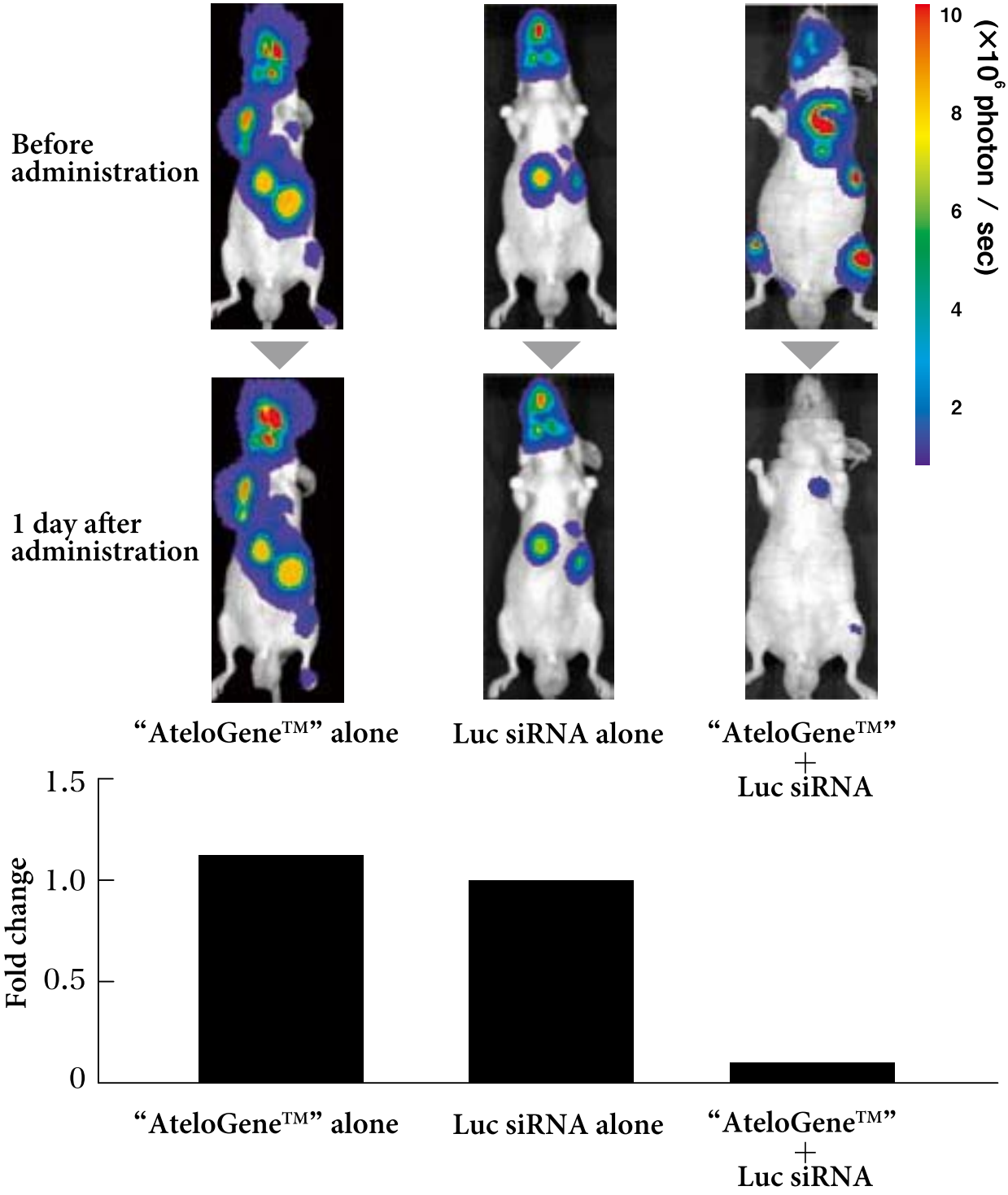




When luciferase siRNA (Luc siRNA) was administered using “AteloGene™” against luciferase-expressing tumor cells that were subcutaneously grafted, the luciferase expression was more inhibited compared with the result observed after the administration of Luc siRNA alone.

【Systemic administration】

Suppression of gene expression of generalized metastasized tumors in mice[3].



When luciferase siRNA (Luc siRNA) was administered using "AteloGene™" against luciferase-expressing generalized metastasized tumor cells, the luciferase expression was more inhibited compared with the result observed after the administration of Luc siRNA alone.

Industrial property rights

Concerning the technique of introducing the nucleic acid component using this kit and collagen, Dainippon Sumitomo Pharma Co., Ltd. and Koken Co., Ltd. has either obtained the patent or the patent is under application (PCT/JP02/06137, patent pending 2002-503340) in Japan and various countries abroad. The usage of this product is limited to tests and research. Other usage of this product may infringe the patent. Please pay attention in this regard.

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