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Catalog No. A1000, A1000S

GoldMAN (For gene transfer)

Store at Room temperature

Protocol of gene transduction using GoldMAN

[Materials]

- Adenovirus vector
- GoldMAN (0.1mg-Fe/mL)
  (Disperse nanoparticles by sonication for few minutes before use)
- Cells (i.e. B16BL6 cell)
- 6, 24 or 96-well plate (Flat bottom)
- Magnetic Plate (OZ BioScience, France)
- CO_2 incubator (37°C, 5% CO_2)

[Protocol]

Day 1:

Preparation of cells

1. Plate B16BL6 cells in growth medium (see table 1).
2. Culture for 12-24 hrs in the CO_2 incubator.

Table 1 The number of B16BL6 cell and the volume of growth medium in each plate.

Plate	Number of plated cells	Volume of growth medium
6-well plate	1.2×10^5 – 2.5×10^5	2500 µl/well
24-well plate	2.5×10^4 – 5.0×10^4	500 µl/well
96-well plate	5.0×10^3 – 1.0×10^4	100 µl/well

Day 2:

Preparation of Ad/GoldMAN complex

3. Dilute Ad solution at 2.0×10^7 vp/µl in growth medium.
4. Mix Ad solution and GoldMAN solution (see table 2), and then incubate it for 5 min at room temperature.

Table 2 The volume of Ad solution and GoldMAN solution.

Plate	Volume of Ad solution	Volume of GoldMAN solution
6-well plate	125 µl/well	125 µl/well
24-well plate	25 µl/well	25 µl/well
96-well plate	5 µl/well	5 µl/well

Transfection

5. Place the cells in the plate on Magnetic Plate.
6. Add Ad/GoldMAN complex to each well (see table 2) and culture for 1 hr in the CO_2 incubator.
7. Remove the plate from Magnetic Plate, and then culture the plate for 24 hrs in the CO_2 incubator.

Day 3

Gene expression

8. Assess the gene expression of Ad.

[Reference]

1. Direct cell entry of gold/iron-oxide magnetic nanoparticles in adenovirus mediated gene delivery. Kamei K, Mukai Y, Kojima H, Yoshikawa T, Yoshikawa M, Kiyohara G, Yamamoto TA, Yoshioka Y, Okada N, Seino S, Nakagawa S. ***Biomaterials***. 2009, 30(9):1809-14.
2. Functionalization of magnetic gold/iron-oxide composite nanoparticles with oligonucleotides and magnetic separation of specific target. Kinoshita T, Seino S, Mizukoshi Y, Nakagawa T, Yamamoto TA., ***J Magn Magn Mater.***, 2007, 311:255-258.
3. Synthesis of gold/magnetic iron oxide composite nanoparticles for biomedical applications with good dispersibility. Seino S, Kusunose T, Sekino T. Kinoshita T, Nakagawa T, Kakimi Y, Kawabe Y, Iida J, Yamamoto TA, Mizukoshi Y. ***J Appl Phys.***, 2006, 99:08H101

o **Application**

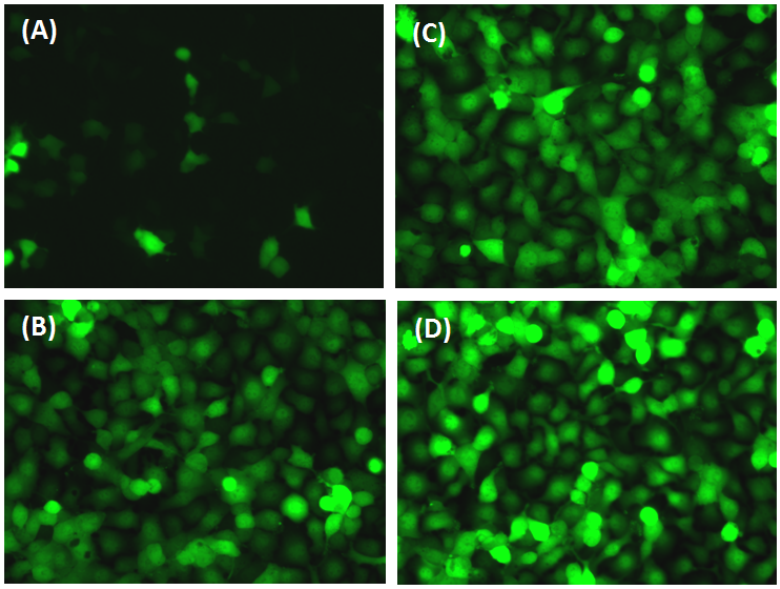

Figure 1 Gene transduction of the Ad/GoldMAN complex into B16BL6 CAR (-) cells.
Efficient gene transfer by Ad/GoldMAN complex was assessed by 1.0 x 10^8 vp of Ad-EGFP. Gene expression of EGFP in B16BL6 cells was observed under fluorescence microscopy. (A) Transfection using Ad-EGFP/GoldMAN complex without magnetic force. (B)-(D) Transfection using Ad-EGFP/GoldMAN complex under magnetic force for (B) 15 min, (C) 30 min, (D) 60 min.

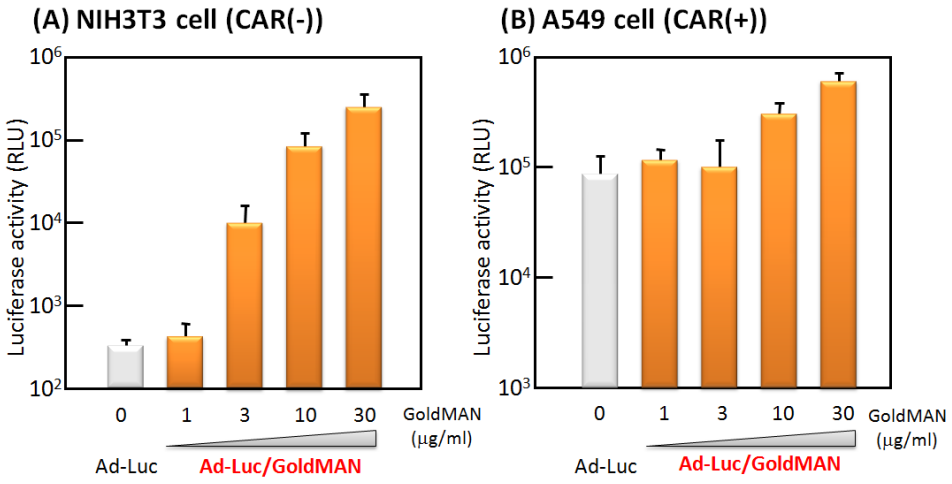

Figure 2 Gene transduction of the Ad/GoldMAN complex into NIH3T3 and A549 cells.
Efficient gene transfer by Ad/GoldMAN complex was assessed by 1.0 x 10^8 vp of Ad-Luc (Luciferase expressing Ad). (A) NIH3T3 CAR(-) cell, (B) A549 CAR (+) cell.

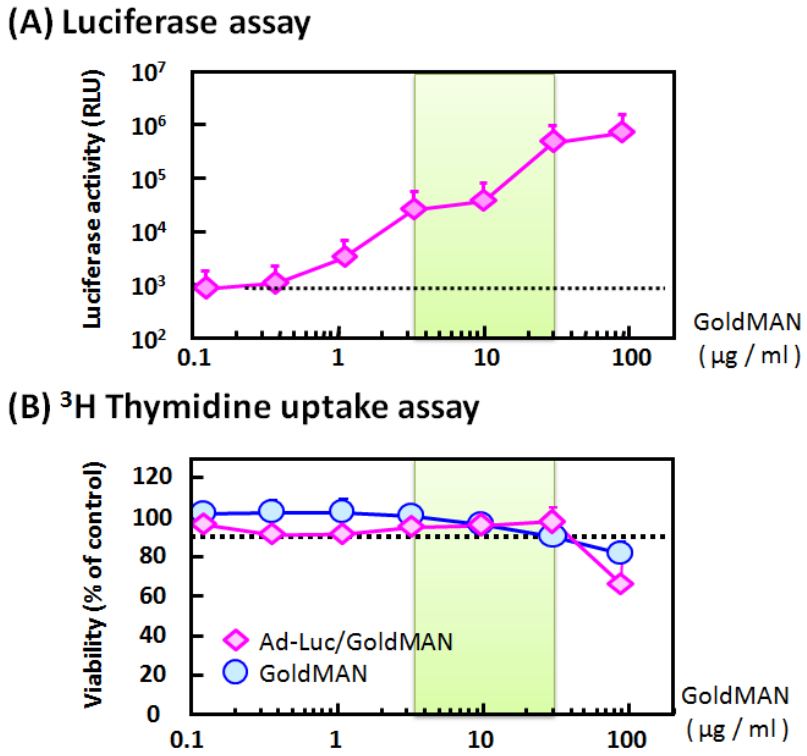

Figure 3 Optimization of GoldMAN in terms of both transduction efficiency and safety.
(A) Quantitative gene expression of Ad/GoldMAN was assessed using 1.0 x 10^8 vp of Ad-Luc. Serially-diluted GoldMAN was mixed with Ad-Luc, and luciferase expression (RLU, relative light units.) was determined using a commercial assay system. Each bar represents the mean ± SD. Shaded region represents optimal conditions in terms of both transduction efficiency and safety. (B) The cytotoxicity of Ad/GoldMAN was assessed by [^{3}H]-thymidine incorporation. Assay using a method similar to that described in Fig. 2A. Each bar represents the mean ± SD. (♦) GoldMAN-treated cells (○) Ad/GoldMAN-treated cells. Shaded region represents optimal conditions in terms of both transduction efficiency and safety.

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