

Use of Ab-Carrier™ to introduce GFP antibody and GFP antigen into HeLa cells

Experimental protocol

Seed HeLa cells in a 12-well plate

(1.0×10^5 cell/well; medium volume MEM (+ 10% FBS) 1 mL/well)

↓ 37° C in the presence of 5% CO₂ for 24 hours

Add anti-GFP antibody to GFP solution (antigen-antibody complex formation)

| rGFP (0.2mg/mL) | Anti-GFP (0.2mg/mL) | PBS | Total Volume |
|-----------------|---------------------|------|--------------|
| 1 μL | 10 μL | 9 μL | 20 μL |

↓ Room temperature for 60 minutes

Add 1 μL of Ab-Carrier™ and mix well

↓ room temperature for 20 minutes

Add 21 μL/well of reaction solution to HeLa cells after 24-hour culture

↓ 37° C in the presence of 5% CO₂ for 4 hours

Remove medium and wash with PBS 1 mL/well × 2 times

↓

Confocal laser microscope observation

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Add 0.25% Trypsin-EDTA 100 μL/well

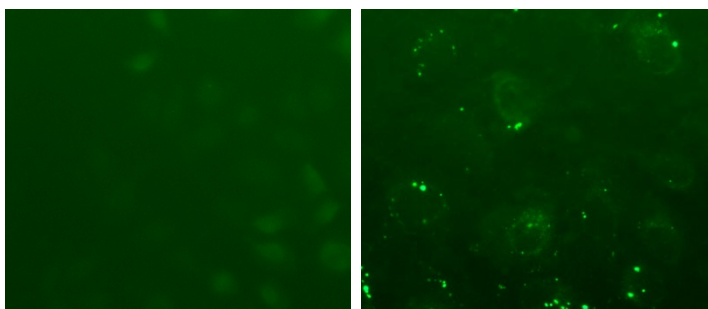
↓ 37° C in the presence of 5% CO₂ for 2 minutes

Add 1 mL of MEM (+ 10% FBS)

↓

FACS analysis (CYTOMICS FC 500; Beckman)

Figure 1. Confocal laser microscope image



Ab-Carrier™ (-)

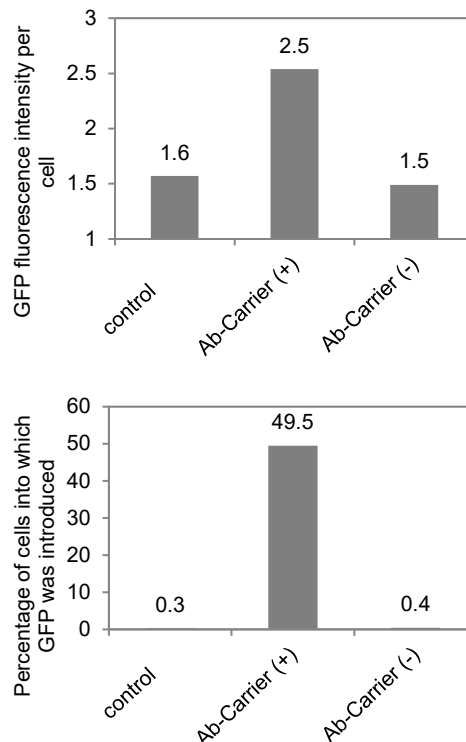
Ab-Carrier™ (+)

(Data provided by Yasuo Shinohara, Tokushima University)

- GFP (antigen protein)
(0.2 mg/mL; Cat No. MB-0752; Funakoshi)
- Anti-GFP antibody (rabbit polyclonal IgG)
(0.2mg/mL; Cat No.29779, Funakoshi)

* Used after removing the preservative (NaN₃) by desalting and replacing the solvent with PBS.

Figure 2. FACS analysis results



GFP and anti-GFP antibody (rabbit IgG) were mixed, reacted at room temperature for 1 hour, and reacted with Ab-Carrier™ at room temperature for 20 minutes. After adding the reaction solution to the cells and incubating at 37° C in the presence of 5% CO₂ for 4 hours, fluorescence was observed with a confocal laser microscope. The GFP fluorescence intensity per cell and the ratio of cells into which GFP was introduced were evaluated by FACS. GFP was introduced into cells together with antibody and GFP fluorescence was observed inside the cells. The introduction of GFP into cells was also confirmed by FACS. From these results, it was confirmed that Ab-Carrier™ can introduce not only antibodies but also antigen molecules into cells.

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