

## Ab-Carrier™

# Species-specific IgG transfection efficiency into HeLa cells

### Experimental protocol

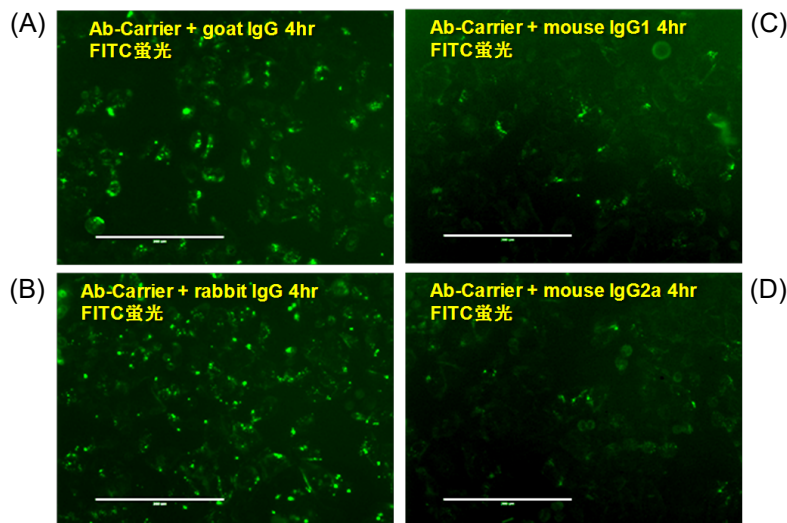
Seeding HeLa cells in a 12-well plate  
( $1.0 \times 10^5$  cell/well; medium volume MEM (+ 10% FBS) 1 mL/well)  
↓ 37° C in the presence of 5% CO<sub>2</sub> for 24 hours  
Ab-Carrier™ 1 μL was added to 20 μL of FITC-labeled IgG (0.1 mg/mL) and mixed well.  
↓ room temperature for 20 minutes  
Add 21 μL/well of reaction solution to HeLa cells after 24-hour culture  
↓ 37° C in the presence of 5% CO<sub>2</sub> for 4 hours  
Remove the medium and wash with PBS 1 mL/well × 2 times  
↓  
Fluorescent microscope observation (EVOS digital inverted microscope; LMS)  
↓  
Add 0.25% Trypsin-EDTA 100 μL/well  
↓ Incubation at 37° C in the presence of 5% CO<sub>2</sub> for 2 minutes  
Add 1 mL of MEM (+ 10% FBS)  
↓  
FACS (CYTOMICS FC 500; Beckman)

**Table 1. Antibody transfer activity (evaluated by FACS)**

Upper row: Ratio of cells into which antibody was introduced (%)

Lower row: Amount of antibody introduced per cell (Relative fluorescence intensity)

Goat polyclonal IgG	93.2%
	14.0
Rabbit polyclonal IgG	92.6%
	17.9
Mouse monoclonal IgG1	81.1%
	3.6
Mouse monoclonal IgG2a	78.1%
	4.8



**Figure 1. Fluorescence microscope image of HeLa cells 4 hours after introduction of FITC-labeled antibody**

(A) Goat polyclonal IgG  
(B) Rabbit polyclonal IgG  
(C) Mouse monoclonal IgG1  
(D) Mouse monoclonal IgG2a  
Green: FITC labeled antibody  
Scale bar: 200 μm

HeLa cells ( $1.0 \times 10^5$  cells/mL) 24 hours after seeding in a 12-well plate were treated with a mixture of antibody transfection reagent Ab-Carrier™ and FITC-labeled IgG antibodies (goat, rabbit, mouse (IgG1, IgG2a)). The cells were cultured for 4 hours and then observed with a fluorescence microscope (phase difference). Fluorescence was observed in most cells (Fig. 1). The proportion of cells into which the antibody was introduced (antibody introduction rate) and the amount of antibody introduced per cell (relative fluorescence intensity) were evaluated by FACS. Goat/rabbit IgG showed a high antibody transfer rate of 90% or more, mouse IgG1 of 80% or more, and mouse IgG2a of 75% or more.