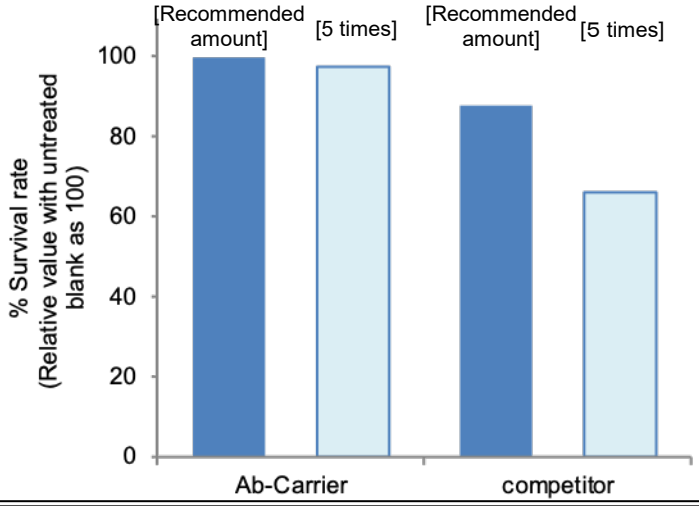
Ab-Carrier™ cytotoxicity evaluation Using the WST-1 proliferation assay

Experimental protocol

Seeding HeLa cells in 96-well plate
(5.0 × 10^3 cell / well; medium volume MEM (+ 10% FBS) 0.1 mL/well)
↓ 37° C in the presence of 5% CO2 for 24 hours
Add 1 µL of Ab-Carrier™ to 20 µL of control IgG (goat; 0.1 mg/mL) and mix well.
↓ room temperature for 20 minutes
Add reaction mixture to HeLa cells after 24-hour culture
[Recommended amount] 1.5 µL/well
[5 times the amount] 7.5 µL/well
↓ 37° C in the presence of 5% CO2 for 24 hours
Remove the medium and wash with PBS 1 mL/well × 2 times
↓
Add 110 µL of WST-1 reagent (MEM medium: pre-mix WST-1 = 10: 1)
↓ 37° C in the presence of 5% CO2 for 2 hours
Measure A 450nm (reference; A 630nm)

WST-1 Cell Proliferation/Viability Assay Reagent:
Premix WST-1 Cell Proliferation Assay System (Takara Bio Inc.)



%Survival rate
(Relative value with untreated blank as 100)

	[recommended amount]	[5 times]
Ab-Carrier™	100	97.9
Competitor	87.8	66.1

The mixture of antibody transfection reagent Ab-Carrier™ and goat IgG was added to HeLa cells (5.0 x 10^4 cells/mL) 24 hours after seeding in a 96-well plate, and cell viability after 24-hour culture was measured using the WST-1. Cell viability 24 hours after the addition of Ab-Carrier™ showed a high value of 97.9% even when 5 times the recommended amount was added. On the other hand, for cells to which competitors were added, the survival rate decreased to 87.8% and the 5-fold amount decreased to 66.1%. Thus, Ab-Carrier™ exhibits minimal cytotoxicity even when added at 5 times the recommended amount.

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