

ProteoCarry™ <Protein transfection reagent>

Catalog NO. FDV-0015

Research use only, not for human or animal therapeutic or diagnostic use

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Product Background

ProteoCarry™ is a novel peptide-based transfection reagent for exogenous proteins such as functional proteins, peptides and antibodies with highly efficiency of cytosolic delivery. Protein transfection, intracellular delivery of proteins, is a powerful method to analyze cellular response to the protein of interest. While plasmid-based gene expression generally needs 12-24 hours to express proteins well, protein transfection reagents can immediately import functional proteins into living cells within several hours. Although many protein transfection reagents including peptide-, cationic lipid- and polymer-based compounds have been developed to date, these reagents struggle to deliver proteins into “cytosol”. Commonly these reagents interact with protein to form complexes and subsequently protein-reagent complexes are entered into cells via endocytosis pathway. But protein-reagent complexes are able to escape endosomes with little efficiency and consequently transported to lysosomes to be degraded. Highly effective endosomal escape of proteins is the most important subject of protein transfection reagents.

ProteoCarry™ can overcome this problem based on a novel and potent pH-dependent endosomal membrane-lytic activity. Proteins and ProteoCarry™ can be delivered into endosomes by endocytosis pathway and also escape to cytosol by its highly membrane-lytic activity with low cytotoxicity.

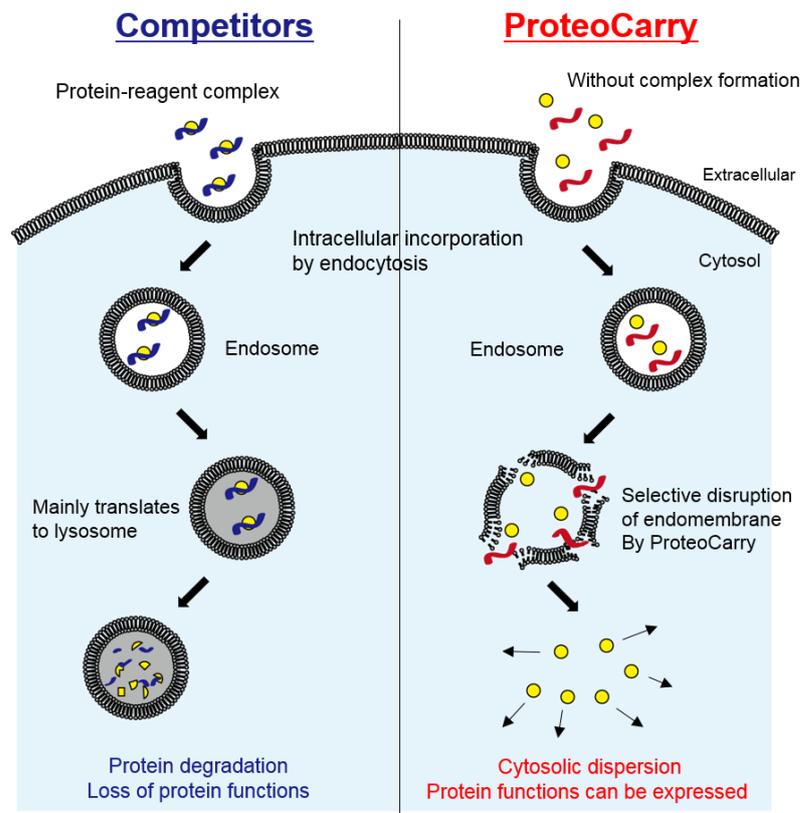


Figure 1. The principle of ProteoCarry™

Left: Problems of conventional competitors

Right : Principle of ProteoCarry™

Description

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Kit components

- ProteoCarry™, 4mg in vial (**vial A**)
- FITC-dextran for positive control, 2 mg in vial (**vial B**)

Solubility: ProteoCarry™, soluble in water

FITC-dextran, soluble in water

Assay number:

Each reagent is enough to perform following assay numbers under the standard protocol below.

ProteoCarry™		FITC-dextran	
6 well scale	14 assays	6 well scale	5 assays
12 well scale	28 assays	12 well scale	10 assays
24 well scale	56 assays	24 well scale	20 assays
48 well scale	140 assays	48 well scale	50 assays
96 well scale	280 assays	96 well scale	100 assays

Application examples

- Intracellular delivery of exogenous or recombinant proteins including peptides, enzymes, antibodies
- Intracellular delivery of bio-macromolecules including glycosaminoglycans etc.

Storage

Storage (powder) : Store powder at -20°C

Storage (solution) : After reconstitution in water (please read “How to use”), aliquot and store at -20°C.

Avoid repeated freeze-thaw cycles. For FITC-dextran protect from light.

Validated cell types

HeLa, SW280, COS7, NIH3T3, HUVEC

How to use

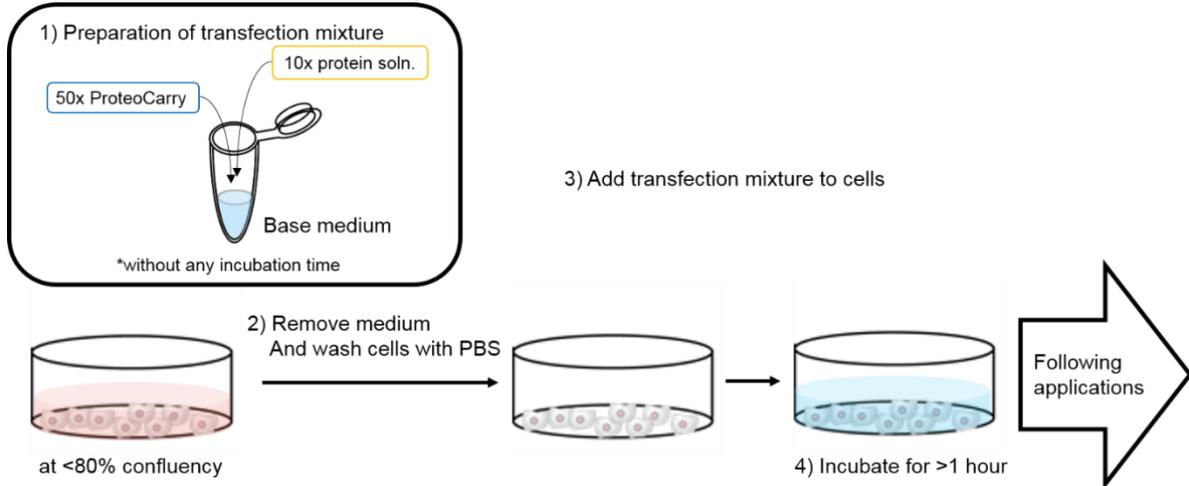


Figure 2. Overview of procedure

Preparation of 50x ProteoCarry™ stock solution

1. Add 581 μl of sterile ultrapure water to **vial A** and mix gently well to be 50x concentrated solution.
2. Stock solution should be stored at -20°C for up to 6 months in small aliquots to avoid repeated freeze and thaw cycles.

Preparation of positive control

1. Add 1 mL of sterile PBS to **vial B** and mix gently well to prepare 10x stock solution (2 mg/ml).
2. Stock solution should be stored at -20°C with protecting from light.

<Memo>

FITC-dextran in this kit is a non-fixable form dextran. Control experiments using FITC-dextran should be on live cells, not on PFA or methanol-fixed cells. After cell fixation, FITC-dextran may be leaked from cells.

Preparation of protein solution

1. Prepare 10x concentrated protein solution with sterile PBS. 10x solution should be prepared at time of use. Please refer Table 1 as a suggestion of protein concentrations.
2. If it is challenging to prepare 10x concentrated solution, please optimize Table 2 below.

Table 1 Suggested concentration of proteins

	Final conc.	10x conc.
Antibody	50-250 $\mu\text{g/ml}$	500-2500 $\mu\text{g/ml}$
Proteins		
saponin	1-10 $\mu\text{g/ml}$	10-100 $\mu\text{g/ml}$
Cre	10-100 $\mu\text{g/ml}$	100-1000 $\mu\text{g/ml}$
Positive control		
FITC-dextran	200 $\mu\text{g/ml}$	2 mg/ml

<Memo>

To observe cytosolic disperse of fluorescent signal from fluorophore-labelled proteins such as FITC-IgG, high concentration of proteins may require. Under the low concentration of fluorophore-labelled proteins, fluorescent signal from endosome may be higher than the signal from cytosol.

Transfection protocol

CAUTION: Following protocol is an example for the positive control experiment, FITC-dextran. Transfection efficiency is highly affected by protein's profile such as molecular weight and net charge, cell-type, and confluency of cells. To obtain best results, please optimize condition for your experiments.

Cell preparation

Cells are seeded and allowed to reach up to 80% confluency in 24 hours.

<Memo>

Confluency of cultured cells may be influence on transfection efficiency. Please optimize cell number for each experiment.

Transfection

1. Mix reagents according to the following table (= Transfection mixture).

Table 2 Suggested amount of transfection mixture

Scale	50x ProteoCarry™ (μL)	10x Protein soln. (μL)	Base medium volume (μL)	Total volume (μL)
96 well	2	10	88	100
24 well	10	50	440	500
12 well	20	100	880	1000
6 well	40	200	1760	2000

<Memo>

You can choice serum-free, serum-supplemented media or PBS as the basal medium of protein transfection. Delivery efficiency through ProteoCarry™ is not affected by serum (<10% FBS). In some cases, PBS give the best results. Please select a basal medium according to your cells of tested.

2. Remove culture medium, wash cells with PBS twice and add the transfection mixture prepared above.
3. Culture cells in the transfection mixture at 37°C for at least 1 hour. The incubation time for the best delivery efficiency is depends on proteins. Please optimize incubation time.
4. Remove culture medium, wash cells with PBS twice and add fresh medium containing serum and culture cells for appropriate time for your experiments. Cells can be used for following applications.

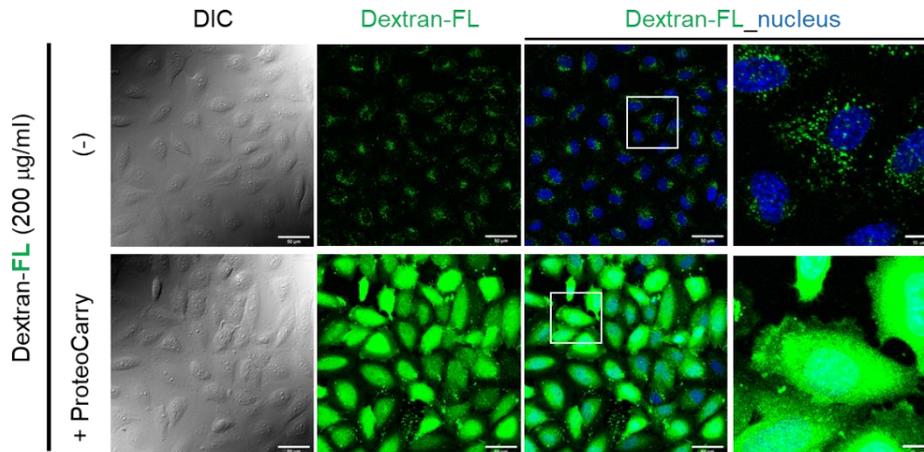
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Mechanism of intracellular delivery of proteins by ProteoCarry™ is based on endocytosis and macropinocytosis pathways. Please avoid to use inhibitors for endocytosis and/or macropinocytosis pathway during transfection.

Application data

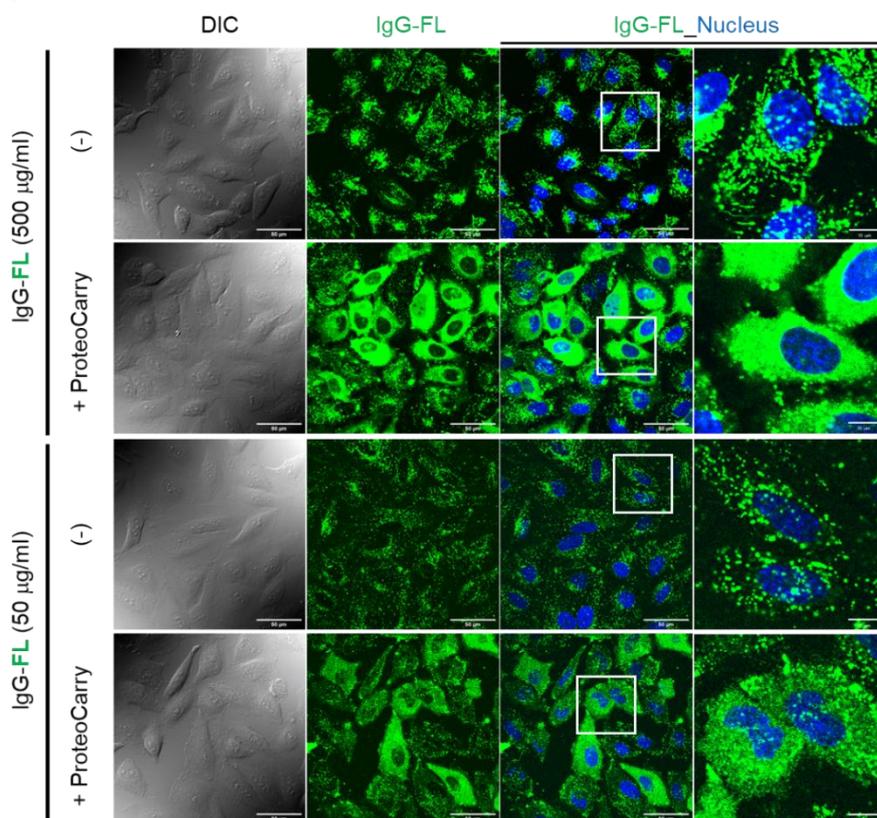
Transfection of fluorophore-conjugated dextran

HeLa cells were treated with green fluorophore-conjugated dextran (Dextran-FL, average MW 10 kDa, final 200 $\mu\text{g/ml}$) in the presence or absence of ProteoCarry™ (1x conc.) for 1 hour at 37°C. Without ProteoCarry™, fluorescent signals were detected from endosome-like dot structures inside cells. On the other hand, ProteoCarry™ clearly stimulated cytosolic delivery of Dextran-FL.



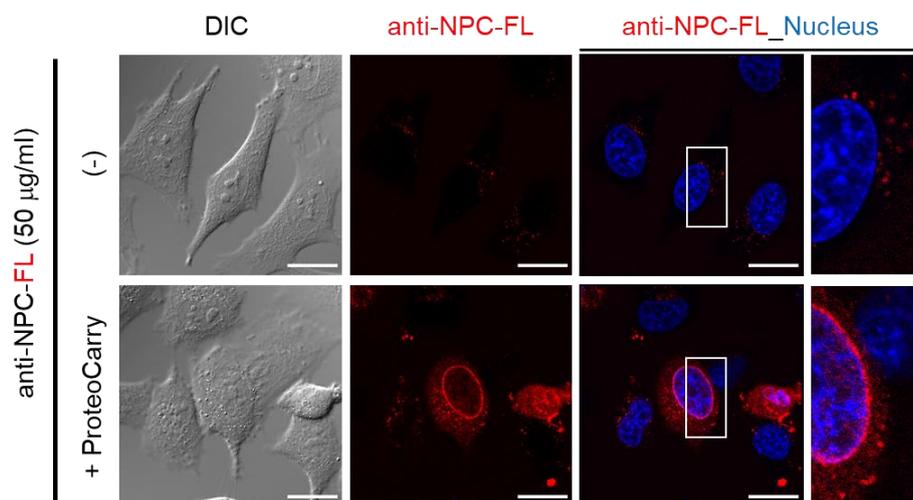
Transfection of fluorophore-conjugated IgG

HeLa cells were treated with green fluorophore-conjugated IgG (IgG-FL, final 50 or 500 $\mu\text{g/ml}$) in the presence or absence of ProteoCarry™ (1x conc.) for 1 hour at 37°C. Without ProteoCarry™, IgG-FLs were aggregated into endosome-like dot structures. On the other hand, ProteoCarry™ promoted cytosolic delivery of IgG-FL even if some IgG-FLs were detected in endosomes.



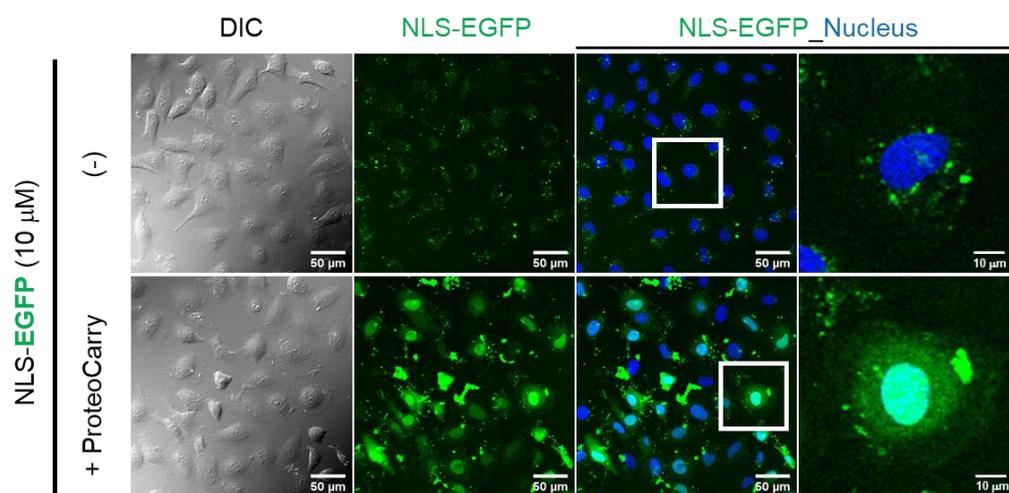
Transfection of fluorophore-conjugated anti-nucleus pore complex (NPC)

HeLa cells were treated with red fluorophore-conjugated anti-nucleus pore complex (NPC) antibody (anti-NPC-FL, final 50 $\mu\text{g/ml}$) in the presence or absence of ProteoCarryTM (1x conc.) for 1 hour at 37°C. Without ProteoCarryTM, a little of fluorescent signal was observed in dot-like structure. On the other hand, ProteoCarryTM clearly promoted localization of anti-NPC-FL to peri-nuclear structure. This result indicates that ProteoCarryTM could induces incorporation of anti-NPC-FL into cytosol and binding of antibody to nucleus pore complex.



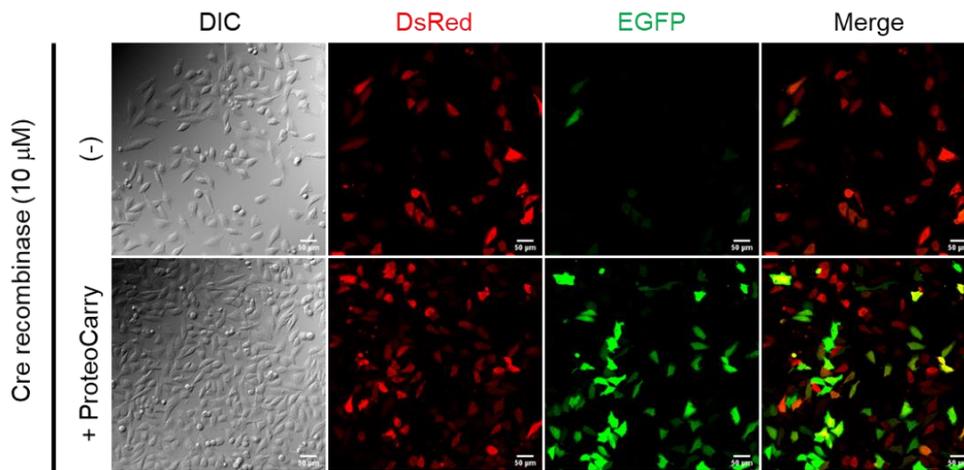
Transfection of nuclear-localizable EGFP

HeLa cells were treated with nuclear localization signal (NLS)-tagged EGFP (NLS-EGFP, final 10 μM) in the presence or absence of ProteoCarryTM (1x conc.) for 1 hour at 37°C. Without ProteoCarryTM, a little of fluorescent signal was observed in endosome-like dot structures. On the other hand, ProteoCarryTM promoted nuclear-localization of NLS-EGFP.



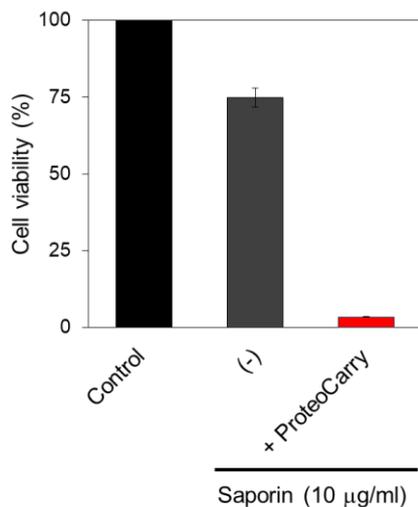
Transfection of Cre recombinase for Cre/lopX recombination assay

HeLa cells were transfected with a plasmid which encodes loxP-DsRed-stop-loxP-EGFP sequence by conventional lipofection reagent. Next day, the HeLa cells were treated with recombinant Cre recombinase (final 10 μ M) for 1 hour at 37°C with or without ProteoCarry™ (1x conc.). Without ProteoCarry™, almost cells still keep DsRed expression under the treatment of Cre recombinase. On the other hand, ProteoCarry™ promoted conversion from DsRed to EGFP by Cre/loxP recombination.



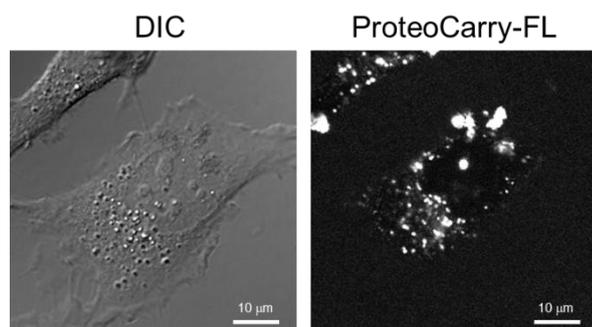
Transfection of Saporin enzyme

Saporin is an enzymatically cytotoxic protein which binds to ribosomes and inactivates ribosomes via its RNA glycosidase activity. RAW264.7 cells were treated with saporin (final 10 μ g/ml) with or without ProteoCarry™ for 1 hour at 37°C. After 24 hours culture, cell viability was measured by MTT assay. Without ProteoCarry™, saporin induced cell death with less than 30% efficiency. On the other hand, ProteoCarry™ dramatically increased cytotoxicity of saporin and almost cells were dead (>90%).



Reference data: Intercellular localization of FL-labeled ProteoCarry™

To monitor intracellular localization of ProteoCarry™, fluorophore-labeled ProteoCarry™ (ProteoCarry™-FL) was prepared and HeLa cells were treated with ProteoCarry™-FL. Fluorescent signal was observed by confocal microscopy. Signal was detected mainly in endosomal dot like structure and little fluorescent signal was observed in cytosol. This data indicates ProteoCarry™ keeps binding to endosomal membrane after lysis of endosomes.



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