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FRONTIERS IN LIFE SCIENCE

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ProteoCarry <Protein transfection reagent>

Product Background

ProteoCarry is a novel peptide-based transfection reagent for exogenous proteins such as functional proteins 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.

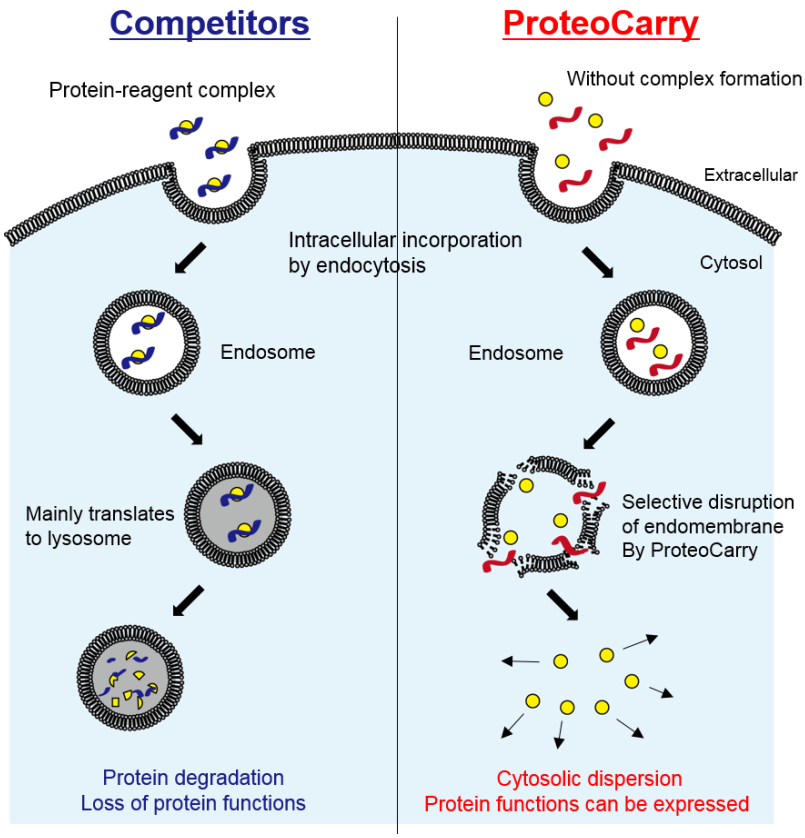


Figure 1. The principle of ProteoCarry
Left: Problems of conventional competitors,
Right : Principle of ProteoCarry

Kit components

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

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

ProteoCarry	
6 well scale	14 assays
12 well scale	28 assays
24 well scale	56 assays
48 well scale	140 assays
96 well scale	280 assays

FITC-dextran	
6 well scale	5 reactions
12 well scale	10 reactions
24 well scale	20 reactions
48 well scale	50 reactions
96 well scale	100 reactions

Storage

Shipping condition : Room temperature
Store : -20ºC

Validated cell types

HeLa, SW280, COS7, NIH3T3, HUVEC

How to use

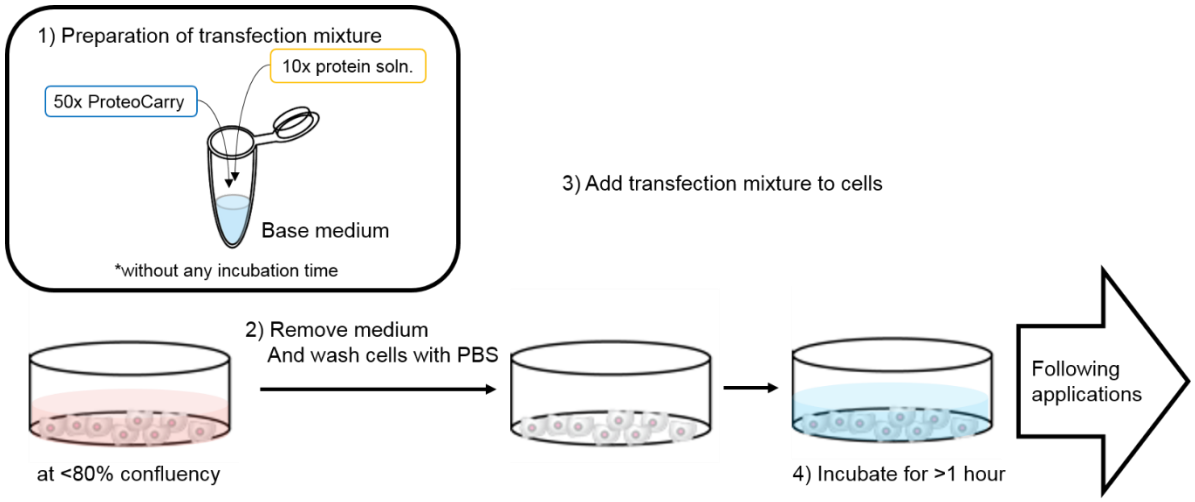


Figure 2. Overview of procedure

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 -20ºC 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 -20ºC.

Preparation of protein solution

1. Prepare 10x concentrated protein solution with sterile PBS. 10x solution should be prepared at time of use.
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 μg/ml	500-2500 μg/ml
Proteins		
saponin	1-10 μg/ml	10-100 μg/ml
Cre	10-100 μg/ml	100-1000 μg/ml
Positive control		
FITC-dextran	200 μg/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 fluorescent 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 cell. 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 37ºC 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.

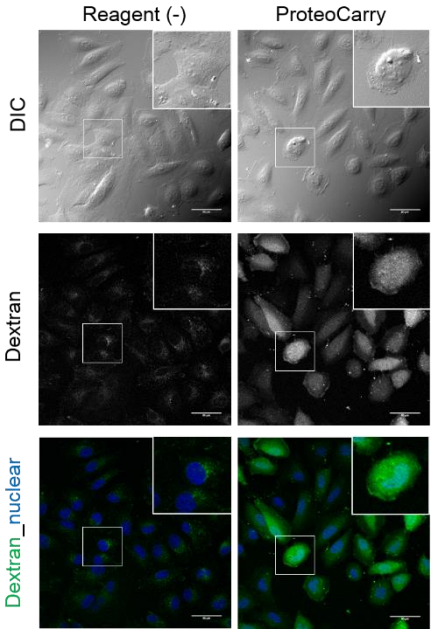
<Memo>

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

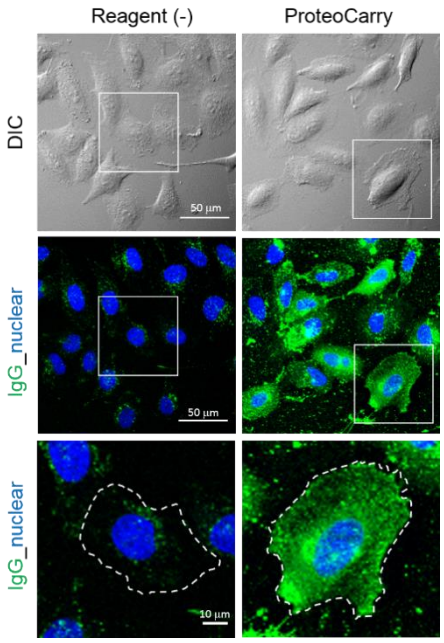
1. FITC-dextran (positive control)

Cell: HeLa (80% confluency)
Cargo : FITC-dextran (200 μg/ml)
ProteoCarry : 1x concentration
Transfection time : 1 hour



2. Fluorofore-labeled IgG

Cell: HeLa (80% confluency)
Cargo : Fluorofore-labeled IgG (250 μg/ml)
ProteoCarry : 1x concentration
Transfection time : 1 hour



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