ProteoJuice™ Protein Transfection Reagent

Description

ProteoJuice Protein Transfection Reagent	0.125 ml	71281-3
	4 × 0.125 ml	71281-4

ProteoJuice Protein Transfection Reagent is an effective reagent for the introduction of intact functional protein into mammalian cells with minimal toxicity and broad cell specificity. ProteoJuice forms non-covalent interactions with protein and has endosome protective properties ensuring delivery of intact protein within the cell. ProteoJuice is compatible with delivery of peptides and small proteins (histones, ~11 kDa), large proteins (antibodies, ~150 kDa) and even multimeric protein complexes such as the β-galactosidase tetramer (~465 kDa) (1).

Cell lines successfully transfected with ProteoJuice				
A549	COS-7	HepG2	MCF-7	PC12
BHK-21	CV-1	HEK-293	Neuro2A	Raw 264.7
CHO-K1	HeLa	L6	NIH-3T3	

Components

0.125 or 4 × 0.125 ml ProteoJuice Protein Transfection Reagent

Storage

Store ProteoJuice tightly capped at 4°C.

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General Considerations and Optimization

General Considerations

- Protein purity will impact protein transfection efficiency. Use only high quality protein.
- Passage cells regularly (e.g., every 2–3 days) and avoid confluent growth. For transfection, use only rapidly proliferating cells. Conditions for cell growth and density should be consistent for optimum reproducibility.
- Serum must be removed from cells prior to protein transfection.

Transfection of Protein	Tissue Culture Format					
	Plate format (wells/plate)			Chamber slide format		
	24	12	6	4	2	1
Number of adherent cells seeded($\times 10^5$)	0.6–1.2	1.0–1.5	2–4	0.6–1.2	1.0–1.5	2–4
Number of suspension cells ($\times 10^5$)	5–7.5	10–15	20–30	5–7.5	10–15	20–30
Volume of complete growth medium in the well or dish (ml)	0.5	1	3	0.5	1	3
Volume of serum-free medium in the transfection mixture (µl)	25	50	100	25	50	100
Volume of ProteoJuice Protein Transfection Reagent (µl)*	1.25	2.5	5	1.25	2.5	5
Amount of purified protein (µg)	1.25	2.5	5	1.25	2.5	5
Volume of serum-free medium added to transfection mixture after incubation (µl)	225	450	900	225	450	900

*When transfecting peptides, reduce the volume of ProteoJuice in half to keep cell toxicity to a minimum.

Optimization

Determining ideal conditions for optimum transfection efficiency with ProteoJuice Protein Transfection Reagent is important for best results. Optimization is suggested for every new combination of cell line and protein. Parameters to consider are cell density, protein concentration, and length of cell exposure to protein transfection mixture.

Cell density

The optimum cell density for transfection is normally between 80–90% confluency for adherent cells and 2–3 $\times 10^6$ cells/ml for suspension cells. The optimum cell density should be determined for every new cell line and should be kept constant for all experiments to ensure reproducibility. Cell density also will influence the optimum quantities of protein used per well.

Protein concentration

We recommend trying to use protein at 1 mg/ml concentration. We recommend using a total of 1.25 µg of protein per well in a 24-well plate as a starting point. Further optimization can be done by altering the protein amount. If a small amount of protein is to be used, such as in the introduction of a toxic protein, additional non-interfering protein should be added as a carrier.

Transfection time

The length of time that the cells are exposed to the transfection mixture will influence transfection efficiency. The optimal protein delivery times should be determined empirically. Generally peptides and small proteins will be delivered into the cell within 2 hours. Most enzymes and moderately sized proteins will transfer into the cells within 3 to 3.5 hours. Large proteins (antibodies) or multimeric proteins may take 5 hours or more to be efficiently delivered.

Note: *With some proteins, incubating the cells for an additional 24 hours in complete medium may increase transfection efficiency.*

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Transfection Procedure

The following procedures describe methods for protein transfection of adherent and suspended eukaryotic cells in a 24-well plate format. Alternative formats require adjustment of the amount of ProteoJuice Protein Transfection Reagent, cell seeding densities and amount of protein (see table above). These protocols are suitable for a range of cell types, but may require optimization for individual cell lines and/or proteins. Refer to *Optimization* for more details. The protein to be transfected should be at a concentration of 1 mg/ml; if the protein concentration is lower, decrease the volume of serum-free medium in the transfection mix to compensate for the larger volume of protein.

Transfection of adherent cells

1. The day before transfection, plate 0.6–1.2 × 10^5 cells in complete growth medium per well of a 24-well plate. Incubate at 37°C (5% CO_2) overnight. Cells should be 80–90% confluent before transfection.
2. For each well to be transfected, place 25 µl serum-free medium (for example, DMEM High Glucose or Opti-MEM™) into a sterile microcentrifuge tube.
3. Add 1.25 µg of protein to the serum-free medium, followed by 1.25 µl ProteoJuice. Mix thoroughly by vortexing.

Notes: *For most proteins the optimal ratio of ProteoJuice to protein is 1:1 (v:w), but the ratio may be varied during optimization.*

When using a peptide the optimal ratio of ProteoJuice to peptide is 1:2 (v:w).

4. Incubate at room temperature for 20 min. During the incubation, remove complete medium from the cells and wash cells 3–4 times with serum-free medium.
5. Add 225 µl serum-free medium to the ProteoJuice/protein transfection mixture. Mix by gentle pipeting.
6. Aspirate medium from the cells and add the transfection mixture dropwise to the cells. Distribute the drops all over the surface of the cells and gently rock the dish to ensure even distribution. Do not swirl the plate, as this will concentrate the transfection mixture in the center of the plate.
7. Incubate the cells at 37°C (5% CO_2) 2–29 h (see *Transfection time*).
   Optional: Supplement the transfection mixture with complete medium after 3 h or aspirate transfection mixture from the cells and replace with complete medium.
8. Wash the cells 3–4 times with serum-free medium or PBS prior to assay or visualization. Washing will remove non-transfected protein that might otherwise influence assay results.
9. Perform cell assay, reporter assay, fluorescence visualization, etc.

Transfection of suspension cells

1. The day before transfection, dilute the cells to a density of 0.2–1 × 10^5 cells per ml, so they will be in log phase growth the following day. Incubate the cells 37°C (5% CO_2) overnight.
2. For each well to be transfected, place 25 µl of serum-free medium (for example DMEM High Glucose or Opti-MEM) into a microcentrifuge tube.
3. Add 1.25 µg of protein to the serum-free medium, followed by 1.25 µl ProteoJuice Protein Transfection Reagent. Mix thoroughly by vortexing.

Notes: *For most proteins the optimal ratio of ProteoJuice to protein is 1:1 (v:w), but the ratio may be varied during optimization.*

When using a peptide the optimal ratio of ProteoJuice to peptide is 1:2 (v:w).

4. Incubate at room temperature for 20 min. During the incubations, count the suspended cells and place 7.5 × 10^5 cells in a sterile microcentrifuge tube. Wash the cells 3–4 times by centrifuging at low speed to pellet the cells and resuspending in serum-free medium.
5. Add 225 µl serum-free medium to the transfection mixture. Mix by gentle pipeting.
6. Remove all medium from the cells and add the transfection mixture. Mix by gently pipeting and transfer to one well of a 24-well plate.

7. Incubate the cells at 37°C (5% CO_2) 2–29 h (see *Transfection time*).
   Optional: Supplement the transfection mixture with complete medium after 3 h or aspirate transfection mixture from the cells and replace with complete medium.
8. Wash the cells 3–4 times with serum-free medium or PBS prior to assay or visualization. Washing will remove non-transfected protein that might otherwise influence assay results.
9. Perform cell assay, reporter assay, fluorescence visualization, etc.

Troubleshooting

Symptom	Possible cause	Solution
Low protein delivery	Serum present during formation of ProteoJuice/protein complex	Use only serum-free medium during formation of complex. Effective protein and ProteoJuice complex formation is dependent on the absence of interfering proteins.
	Cell density suboptimal at time of transfection	The optimal cell density should be determined for each cell type. Try higher and lower cell densities.
	Serum present with the cells before addition of the ProteoJuice transfection mix	If cells were grown in presence of serum, wash the cells 3–4 times before adding serum-free medium and transfection mixture.
	Poor protein quality	Prepare new protein or further purify the existing protein.
High cell toxicity	Incomplete mixing of ProteoJuice/protein complexes with cells	Be sure to thoroughly distribute the transfection mixture to all cells in the plate. The transfection mixture should be added dropwise all across the surface of the medium, and the plate should be rocked back and forth to mix. Do not swirl or rotate the dish, as this may concentrate the ProteoJuice protein complexes in the center of the dish.
	Too little protein in the reaction	Increase the amount of protein in the reaction or add a non-interfering protein as a carrier.
	Cell density too low at time of transfection	The density of the cells at transfection time will depend on the initial plating density, the cell growth rate (doubling time) and the length of time between plating and transfection. Try plating more cells to achieve 80–90% confluency at the time of transfection.

References

1. Hayes, S. (2003) *in**Nova**tions* **17**, 13–16.

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