



ABBIOTEC™

From Biology to Discovery™

Lipodin-Pro™ Protein Transfection Reagent

Cat. No 500100

Cat. No 500110

(version 01-08)

ABBIOTEC
7955 Dunbrook Road, Suite B
San Diego, California 92126, USA
Toll Free: 1 800 854 7453
Telephone: 1 858 586 0500
Fax: 1 858 586 6252
Web: www.abbiotec.com

INSTRUCTION MANUAL

TABLE OF CONTENTS

1. Protein Transfection Technology	1
1.1. Description	1
1.2. Kit Benefits	1
1.3. Kit Contents & Storage	2
1.4. Quality Controls	2
2. Applications	2
2.1. Precautions for Protein Delivery	2
2.2. Cell Types & Targets	3
3. Protocol	3
3.1. Cell Preparation	3
3.2. Protein Transfection Procedure	3
3.3. Protein Transfection using the R-Phycoerythrin Positive Control	4
4. Appendix	5
4.1. Optimization Protocol	5
4.2. Troubleshooting	5
4.3. Related Products	7
4.4. Technical Support	7
4.5. Product Use Limitations	7

1. PROTEIN TRANSFECTION TECHNOLOGY**1.1. Description**

The delivery of proteins inside living cells represents an alternative to nucleic acids transfection and a powerful strategy for functional studies or therapeutic approaches. Several technologies based on the use of peptide transduction domain (PTD) were developed successfully to transduce proteins across the plasma membrane. However, these PTD poorly interact with proteins, and covalent linkage between the protein and PTD is most often required. **Lipodin-Pro™** is a formulation of lipids able to capture proteins through electrostatic and hydrophobic interactions. There is no need for covalent linkage procedure, **Lipodin-Pro™** is directly mixed with the protein of interest for 10 minutes. The mixture is then added to the cells in culture, the lipid-protein complexes are internalized by the cells and the proteins are released into the cytoplasm within few hours without any cytotoxicity. The optimized formulation of **Lipodin-Pro™** is fully biodegradable maintaining a high cell viability upon delivery. The proteins delivered inside the cells with **Lipodin-Pro™** retain both their structure and function whether peptides, proteins or antibodies are used.

1.2. Kit Benefits

Lipodin-Pro™ can be used in various functional studies for cell signaling and apoptotic assays, protein-protein interaction, protein localization and compartment shuttling. When the protein is conjugated to a fluorescent dye, the functional assay can be carried out in living cells under multiple treatments with a single sample.

Principal **Lipodin-Pro™** advantages:

- No need for DNA cloning or nucleic acid transfection
- No chemical ligation or crosslinking
- Serum compatible, no cytotoxicity and biodegradable
- Easier 2-step protocol with ready-to-use reagents
- Deliver functionally active protein within hours
- Higher delivery efficiency with stable cell lines and primary cells

Lipodin-Pro™ successfully delivered numerous proteins in a wide variety of cells: B and R-phycoerythrin, bovine serum albumin, β -galactosidase, human active caspase-3 and various immunoglobulins (unconjugated or labeled with FITC, TRITC, AlexaFluor®488 and AlexaFluor®546). Standard cell lines (CHO, COS-7, HEK-293, HeLa, Jurkat, NIH3T3) and primary cells (neurons, glial cells) were successfully assayed for **Lipodin-Pro™** reagent.

1.3. Kit Contents & Storage

Lipodin-Pro™ protein transfection reagent is offered in 25- and 100-reaction kits, for which a reaction is defined as the amount of reagent required to deliver 2 μ g of R-phycoerythrin positive control protein to cells in one well of a 12-well plate. The number of reactions varies depending on the plate size and type of macromolecule to deliver. Each kit includes the **Lipodin-Pro™** protein transfection reagent, a R-phycoerythrin protein solution (100 μ g/ml in PBS) as positive control, and a comprehensive instruction manual. Kit components are shipped at room temperature and stored at 4°C upon receiving. Components are guaranteed stable for one year.

Catalog No.	Unit	Reagent	Quantity	Storage/Stability
500100	25 rxn	Lipodin-Pro™	0.1 ml	4°C for 1 year
		R-Phycoerythrin	10 μ g	4°C for 1 year
500110	100 rxn	Lipodin-Pro™	0.5 ml	4°C for 1 year
		R-Phycoerythrin	10 μ g	4°C for 1 year

Additional Materials Required:

- Sterile 1.5-ml microcentrifuge tubes
- Sterile PBS, pH7.4
- Serum-free culture medium

1.4. Quality Controls

To assure the performance of each lot of **Lipodin-Pro™ - Protein Transfection Reagent** produced, we qualify each component using rigorous standards. The following assays are conducted *in vitro* to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
<i>Purity</i>	Silica Gel TLC assays. Every compound must have a single spot.
<i>Sterility</i>	Thioglycolate assay. Absence of fungal and bacterial contamination must be obtained for 7 days.
<i>Biological Activity</i>	Delivery of R-Phycoerythrin in NIH3T3 cells monitored by cytofluorimetry and fluorescence microscopy. Every lot must have an acceptance specification of > 80% of the activity of the reference lot.

2. APPLICATIONS

2.1. Precautions for Protein Delivery

Proteins vary in size, structure, composition and activity. Therefore protein delivery conditions with the Lipodin-Pro™ reagent for one particular protein cannot be translated to another protein. We strongly recommend optimizing the protocol using this reagent with your protein of interest. Delivery efficiency also varies between different cell lines, contact us for an updated list of proteins and cells successfully transfected with **Lipodin-Pro™**.

Impurities, contaminants and additives present with your protein of interest affect delivery efficiency. We recommend using a protein sample as pure as possible. We noticed that protein preparations containing high contents of detergents or sodium azide were not compatible with protein transfection, whereas glycerol has no effect.

The instructions given below represent sample protocols that were applied successfully on a variety of cells. Our R&D team has extensively tested and optimized the **Lipodin-Pro™** reagent in order to provide you with the

simplest, straightforward and efficient procedure. Therefore, we recommend starting with our general protocol as guidelines for your first experiments.

2.2. Cell Types and Targets

Lipodin-Pro™ - Protein Transfection Reagent is applicable with numerous cell types and multiple proteins. This reagent has been successfully tested on a variety of immortalized cell lines as well as several primary cells. Contact us for an updated list of proteins and cells successfully transfected with Lipodin-Pro™.

Cell Line	Cell Type	Source
3T6	Embryonic fibroblasts	Mouse
A549	Non-small cell lung carcinoma	Human
B16-F10	Melanoma	Mouse
BEAS-2B	Bronchial epithelial cells	Human
BHK21	Fibroblasts (Kidney)	Hamster
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblasts (Kidney)	Green Monkey
HaCaT	Keratinocytes	Human
HEK-293	Transformed Embryonic (Kidney)	Human
HeLa	Cervical Epithelial Carcinoma	Human
Jurkat	T cell leukemia (lymphoma)	Human
L929	Fibrosarcoma	Mouse
K562	Myelogenous leukemia	Human
MDCK	Epithelial (Kidney)	Canine
N2A	Neuroblastoma	Mouse
NIH3T3	Fibroblasts	Mouse
Raw264.7	Monocytes/macrophages	Mouse
U87	Glioblastoma	Human
Vero 10A1	Epithelial (Kidney)	Monkey
Primary cells		
Neurons		Rat
Glial cells		Rat

3. PROTOCOL

3.1. Cell Preparation

Adherent cells. We recommend seeding the cells the day prior to the protein delivery experiment. The suitable cell density will depend on the growth rate and the viability of the cells. Cells should be 50-80% confluent (percentage of growth surface covered with cells) at the time of experiment. Recommended numbers of cells to seed are provided in the table 1.

Suspension cells. For fast growing cells, split the cells the day before the protein delivery experiment at a density of 2 to 5 x 10⁵ cells/ml.

Table 1: Recommended number of cells to seed.

Culture Dish	Number of Adherent Cells	Number of Suspension Cells	Cell Overlay Volume
96-well plate	0.05 – 0.15 x 10 ⁵	0.5 – 1 x 10 ⁵	100 µl
24-well plate	0.5 – 1 x 10 ⁵	1.5 – 5 x 10 ⁵	400 µl
12-well plate	1 – 2 x 10 ⁵	2.5 - 10 x 10 ⁵	900 µl
6-well plate	2.5 – 5 x 10 ⁵	5 – 20 x 10 ⁵	1.8 ml
60-mm dish	5 – 10 x 10 ⁵	1 – 5 x 10 ⁶	3.8 ml
100-mm dish	12 – 30 x 10 ⁵	2.5 – 10 x 10 ⁶	7.6 ml
T-75 flask	15 – 40 x 10 ⁵	5 – 15 x 10 ⁶	9.6 ml

3.2. Protein Transfection Procedure

The following protocol is based on one well of a 12-well plate seeded with 10⁵ cells one day prior protein transfection. Each well corresponds to one reaction. Recommended amounts of protein and transfection reagent are provided in the table 2.

Table 2: Recommended amount of protein and Lipodin-Pro™ reagent.

Culture Dish	Protein Quantity	Lipodin-Pro™	Dilution Volume	Total Medium Volume
96-well plate	0.4 µg	0.4 µl	20 µl	120 µl
24-well plate	1 µg	2 µl	100 µl	500 µl
12-well plate	2 µg	4 µl	100 µl	1 ml
6-well plate	5 µg	10 µl	200 µl	2 ml
60-mm dish	10 µg	20 µl	200 µl	4 ml
100-mm dish	30 µg	60 µl	400 µl	8 ml
T-75 flask	35 µg	70 µl	400 µl	10 ml

IMPORTANT: Allow kit components to equilibrate at room temperature and vortex 10 seconds at highest setting before use.

- 1) In a sterile 1.5-ml microcentrifuge tube, prepare a diluted solution of the protein to be delivered at 100 µg/ml in a sterile phosphate buffer saline (PBS), pH7.4. Keep the protein solution for step 3 of this protocol.
 - Do not use tissue culture media for this step! We recommend using PBS but other buffer such as Hepes, HBS or Tris can also be used.
 - Low amounts of glycerol (<5% final) do not interfere with protein delivery into cells, whereas BSA inhibits protein delivery. Remove BSA from protein sample before proceeding by using a desalting or dialysis device.
- 2) Transfer 4 µl of Lipodin-Pro™ reagent in a sterile 1.5-ml microcentrifuge tube.
 - Be careful to add the reagent to the bottom of the microcentrifuge tube without touching the wall of the tube, which would result in reagent loss.
- 3) Add 20 µl of protein solution prepared in step 1 (2 µg total) to the tube containing the Lipodin-Pro™ reagent, and mix by pipetting up and down.
- 4) Incubate reaction for 15 min at room temperature.
- 5) Add 100 µl of serum-free medium to the mixture and transfer immediately in the culture dish containing the cells to transfect grown in standard culture medium. Mix gently by pipetting up and down to distribute the mixture evenly.
 - Lipodin-Pro™ reagent can be used with cells cultured in serum-free medium. In this case, replace the complete culture medium by serum-free medium. This procedure can be more efficient to deliver proteins in cells difficult to transfect. After 3-4h, add serum-containing medium if further incubation time is necessary.
- 6) Incubate the cells at 37°C in a tissue culture incubator under standard conditions until evaluation of the protein delivery efficiency. Protein delivery can be observed as soon as 3 hours post-transfection.

3.3. Protein Transfection using the R-Phycoerythrin Positive Control

The following protocol is based on one well of a 12-well plate seeded with 10⁵ cells one day prior protein transfection. Each well is one reaction.

- 1) Transfer 2 µl of Lipodin-Pro™ reagent in a sterile 1.5-ml microcentrifuge tube.
- 2) Add 1 µl of R-Phycoerythrin control (1 µg total) to the tube containing the Lipodin-Pro™ reagent, and mix by pipetting up and down.

- 3) Incubate reaction for 15 min at room temperature.
- 4) Add 100 µl of serum-free medium to the mixture and transfer immediately in the culture dish containing the cells to transfect grown in standard culture medium. Mix gently by pipetting up and down to distribute the mixture evenly.
- 5) Incubate the cells at 37°C in a tissue culture incubator under standard conditions until evaluation of the protein delivery efficiency. Fluorescence emission (575 nm) can be observed directly in the culture dish as soon as 3 hours post-transfection by fluorescence microscopy.

4. APPENDIX

4.1. Optimization Protocol

When the protocol described in paragraph 3 does not deliver satisfying results, we recommend using these steps below to optimize protein transfection into cells using the **Lipodin-Pro™** transfection reagent.

We recommend that you optimize the different parameters starting from the conditions given in the protocol above within the range indicated in the table 3.

- 1) **Optimizing the ratio Lipodin-Pro:protein.** Vary the amount of the **Lipodin-Pro™** reagent using a fixed amount of protein as recommended in the table 3. Example: add 1 µg of protein to 0.5, 1, 2.5 and 5 µl of Lipodin-Pro™ reagent in a 24-well plate seeded with cells.
- 2) **Increasing the amount of protein.** Increase the amount of protein to be delivered maintaining constant the optimized ratio Lipodin-Pro:protein determined in step 1 as recommended in the table 3. Example: if a 1:1 Lipodin-Pro:protein ratio is best, transfect cells with mixtures of 0.5 µl:0.5 µg, 1 µl:1 µg, 2 µl:2 µg Lipodin-Pro:protein amounts in a 24-well plate seeded with cells.
- 3) After having identified the optimal quantities of **Lipodin-Pro™** and protein, you can optimize other parameters such as the cell number and the time course of delivery.

Table 3: Optimization of protein amount and volume of **Lipodin-Pro™** reagent.

Tissue Culture Dish	Protein Quantity	Lipodin-Pro™	Dilution Volume	Total Medium Volume
96 well	0.2 - 0.5 µg	0.2 – 1 µl	20 µl	120 µl
24 well	0.5 - 2 µg	0.5 – 5 µl	100 µl	500 µl
12 well	1 - 4 µg	1 – 10 µl	100 µl	1 ml
6 well	2.5 - 10 µg	2.5 – 25 µl	200 µl	2 ml
60 mm dish	5 - 20 µg	5 – 50 µl	200 µl	4 ml
90 - 100 mm	15 - 60 µg	15 – 120 µl	400 µl	8 ml
T-75 flask	20 - 80 µg	20 – 160 µl	400 µl	10 ml

4.2. Troubleshooting

Problem	Possible Cause	Recommendation
Low delivery efficiency	Lipodin-Pro:protein ratio is not optimal.	Optimize the Lipodin-Pro:protein ratio as indicated in table 3.
	Protein amount delivered to cells is too low.	Use different amount of protein with the optimized Lipodin-Pro:protein ratio as indicated in table 3.
	Protein solution contains contaminants or additives such as BSA or detergents.	Remove contaminants and additives by chromatography, ultrafiltration or dialysis.

	<p>The buffer used to prepare the protein solution does not favor complex formation of the protein with Lipodin-Pro™ reagent. Highly basic proteins are difficult to deliver due to the positively charged residues.</p> <p>Unusual kinetics of transfection due to protein properties. The transfection efficiency can be monitored from 4 hours until 96 hours post-transfection.</p> <p>The Lipodin-Pro™ reagent-protein complexes are aggregated.</p> <p>Cell density is too low or too high. Cell density should be 50-70% confluent at time of transfection for best results.</p> <p>Cells are not healthy or stabilized in culture medium. Cells grown for more than 8 weeks may become resistant to transfection. Cells should be transfected during their exponential growing phase and free of mycoplasma, fungi or bacteria.</p> <p>Culture medium composition is not optimal for transfection. Although serum was not a factor for the transfection of the proteins tested, serum-containing media could impair the transfection of certain proteins for particular cell types.</p>	<p>Change the protein dilution buffer and/or pH to improve the delivery by using Hepes, HBS or Tris buffers. The charge of the protein can be modified with the buffer pH. Only use serum-free medium to prepare the complexes.</p> <p>Monitor protein activity over a longer period of time to identify peak.</p> <p>The mixture of the two components must be prepared and used within 1 hour.</p> <p>Reduce cell density by splitting the cells and wait 24 hours before the transfection experiment. Or, increase cell density by growing cells for one more day before the transfection experiment.</p> <p>Use freshly thawed cells that have been passaged at least once. Contaminated cells should be discarded and a new culture started from a frozen stock.</p> <p>Use serum-free culture medium during the first 4 hours of transfection.</p>
Cellular toxicity	<p>The protein delivered is cytotoxic or triggers cell death.</p> <p>Protein solution contains contaminants or additives that are cytotoxic.</p> <p>Cells are not healthy due to contamination by bacteria, mycoplasma or fungus, or due to incorrect cell density or culture media.</p>	<p>Use suitable controls such as cells alone, Lipodin-Pro™ reagent alone or R-Phycoerythrin positive control provided in the kit.</p> <p>Remove contaminants and additives by chromatography, ultrafiltration or dialysis.</p> <p>Check cells for contamination and start over from a new batch. Check for correct culture medium and cell density at seeding (see table 1 for recommendations).</p>

4.3. Related Products

Lipodin-Ab™ – The Dedicated Antibody Delivery Reagent

Catalog No.	Reagent	Unit
500115	Lipodin-Ab™	25 rxn
500120	Lipodin-Ab™	100 rxn

4.4. Technical Support

If you need assistance with your experiments using this product, please contact our Technical Support Department:

ABBIOTEC

7955 Dunbrook Rd., Ste B

San Diego, CA 92126, USA

Toll Free: 1 800 854 7453

Telephone: 1 858 586 0500

Fax: 1 858 586 6252

Email: techsupport@abbiotec.com

Web: www.abbiotec.com

4.5. Product Use Limitations

Abbiotec, LLC agrees to provide its customers with products under the following terms and conditions. By purchasing and receiving Abbiotec products, customers will agree to the following:

All Abbiotec products are provided solely for research use only. Abbiotec products have NOT BEEN APPROVED FOR HUMAN USE, directly or indirectly. Customers agree not to administer any Abbiotec products to human subjects in any manner or form or to use Abbiotec products for therapeutic or diagnostic purposes. Abbiotec customers will also agree not to use its products commercially without prior consent and without the presence of a signed agreement between customer and Abbiotec, LLC. Licenses for diagnostic, therapeutic, or commercial resale use of Abbiotec products may be available and can only be obtained by contacting Abbiotec directly.

Customers agree to abide and comply by all applicable local and federal laws that govern the use of research products for research use only. This includes the correct use of Abbiotec products as intended and described in the product manual, and the safe handling and disposal of these products according to all applicable governmental regulations.

These product use limitations represent the entire terms and understandings between Abbiotec, LLC and its customers. These use limitations supersede all previous communications, representations, understanding and agreements, whether oral or written, by or between Abbiotec, LLC and the customer regarding the use of Abbiotec products. No change, modification, extension, termination or waiver of any of these use limitations shall be valid unless made in writing and signed by a duly authorized representative of Abbiotec.

Abbiotec trademarks referred to in this publication: From Biology to Discovery™, Lipodin-Ab™, Lipodin-Pro™. “Abbiotec” and the Abbiotec logo are trademarks of Abbiotec, LLC. All content contained in this manual is property of Abbiotec, LLC and may be used only with the expressed written permission of Abbiotec, LLC. All rights reserved.

Trademarks of other companies referred to in this publication: AlexaFluor® (Invitrogen, Inc.).