ABBIOTEC™

From Biology to Discovery™

Lipodin-Ab™
Antibody Delivery Reagent

Cat. No 500115
Cat. No 500120

(version 01-08)

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INSTRUCTION MANUAL

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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-Ab™** is a formulation of lipids optimized to capture antibodies through electrostatic and hydrophobic interactions. There is no need for covalent linkage procedure, **Lipodin-Ab™** is directly mixed with the antibody for 10 minutes. The mixture is then added to the cells in culture, the lipid-antibody complexes are internalized by the cells and the antibody is released into the cytoplasm within few hours without any cytotoxicity. The optimized formulation of **Lipodin-Ab™** is fully biodegradable maintaining a high cell viability upon delivery. The antibodies delivered inside the cells with **Lipodin-Ab™** retain both their structure and function whether they are conjugated or not to an enzyme or dye.

1.2. Kit Benefits

Lipodin-Ab™ can be used in various functional studies for cell signaling and apoptotic assays, protein-protein interaction, protein localization and compartment shuttling. When the antibody 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-Ab ™** advantages:

- Unique reagent dedicated to antibody
- No need for cell fixation protocol
- Serum compatible, no cytotoxicity and biodegradable
- Easy 2-step protocol with ready-to-use reagents
- Deliver functionally active antibody within hours
- Higher delivery efficiency with stable cell lines and primary cells

Lipodin-Ab™ successfully delivered numerous antibodies in a wide variety of cells: monoclonal and polyclonal antibodies from human, mouse, rabbit and rat origins, 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-Ab™** reagent.

1.3. Kit Contents & Storage

Lipodin-Ab™ antibody delivery reagent is offered in 25- and 100-reaction kits, for which a reaction is defined as the amount of reagent required to deliver 2 µg of FITC-labeled IgG positive control antibody to cells in one well of a 12-well plate. The number of reactions varies depending on the plate size and type of antibody to deliver. Each kit includes the **Lipodin-Ab™** antibody delivery reagent, a FITC-labeled IgG solution (100 µg/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
500115	25 rxn	Lipodin-Ab™	0.1 ml	4°C for 1 year
		FITC-labeled IgG	10 µg	4°C for 1 year
500120	100 rxn	Lipodin-Ab™	0.5 ml	4°C for 1 year
		FITC-labeled IgG	10 µg	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-Ab™ - Antibody Delivery 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
Purity	Silica Gel TLC assays. Every compound must have a single spot.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination must be obtained for 7 days.
Biological Activity	Delivery of FITC-labeled IgG 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 Antibody Delivery

Despite conserved properties between antibodies, delivery conditions with the Lipodin-Ab™ reagent for one particular antibody conjugate cannot be translated to another antibody or conjugate. We strongly recommend optimizing the protocol using this reagent with your antibody of interest. Delivery efficiency also varies between different cell lines, contact us for an updated list of antibodies and cells successfully transfected with **Lipodin-Ab™**.

Impurities, contaminants and additives present with your antibody of interest affect delivery efficiency. We recommend using an antibody sample as pure as possible. The presence of stabilizers such as bovine serum albumin (BSA concentration > 0.1 %) inhibits antibody delivery. We strongly recommend removing the BSA using gel chromatography exclusion, protein A/G affinity chromatography or BSA removal kits. We noticed that antibody preparations containing high contents of detergents or sodium azide were not compatible with antibody delivery, 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-Ab™** 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-Ab™ - 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-Ab™.

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
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 antibody 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 10^5 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. Antibody Delivery Procedure

The following protocol is based on one well of a 12-well plate seeded with 10^5 cells one day prior to antibody delivery. Each well corresponds to one reaction. Recommended amounts of antibody and transfection reagent are provided in the table 2.

Table 2: Recommended amount of antibody and **Lipodin-Ab™** reagent.

Culture Dish	Antibody Quantity	Lipodin-Ab™	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 antibody 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 rather than Hepes, HBS or Tris buffers.
- Low amounts of glycerol (<5% final) do not interfere with protein delivery into cells, whereas BSA inhibits antibody delivery. Remove BSA from antibody sample before proceeding by using techniques described in section 2.1.

2) Transfer 4 µl of **Lipodin-Ab™** 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 antibody solution prepared in step 1 (2 µg total) to the tube containing the **Lipodin-Ab™** 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-Ab™** 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 antibodies 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 antibody delivery efficiency. Antibody delivery can be observed as soon as 3 hours post-transfection.

3.3. Antibody Delivery using the FITC-labeled IgG Positive Control

The following protocol is based on one well of a 12-well plate seeded with 10^5 cells one day prior to antibody delivery. Each well is one reaction.

1) Transfer 2 µl of **Lipodin-Ab™** reagent in a sterile 1.5-ml microcentrifuge tube.

2) Add 1 µl of FITC-labeled IgG control (1 µg total) to the tube containing the **Lipodin-Ab™** 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 antibody delivery efficiency. Fluorescence emission (525 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 antibody delivery into cells using the **Lipodin-Ab™** delivery 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-Ab:antibody**. Vary the amount of the **Lipodin-Ab™** reagent using a fixed amount of antibody as recommended in the table 3. Example: add 1 µg of antibody to 0.5, 1, 2.5 and 5 µl of Lipodin-Ab™ reagent in a 24-well plate seeded with cells.

2) **Increasing the amount of antibody**. Increase the amount of antibody to be delivered maintaining constant the optimized ratio Lipodin-Ab:antibody determined in step 1 as recommended in the table 3. Example: if a 1:1 Lipodin-Ab:antibody ratio is best, transfect cells with mixtures of 0.5 µl:0.5 µg, 1 µl:1 µg, 2 µl:2 µg Lipodin-Ab:antibody amounts in a 24-well plate seeded with cells.

3) After having identified the optimal quantities of **Lipodin-Ab™** and antibody, you can optimize other parameters such as the cell number and the time course of delivery.

Table 3: Optimization of antibody amount and volume of **Lipodin-Ab™** reagent.

Tissue Culture Dish	Antibody Quantity	Lipodin-Ab™	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-Ab:antibody ratio is not optimal.	Optimize the Lipodin-Ab:antibody ratio as indicated in table 3.
	Antibody amount delivered to cells is too low.	Use different amount of antibody with the optimized Lipodin-Ab:antibody ratio as indicated in table 3.
	Antibody solution contains contaminants or additives such as BSA or detergents.	Remove contaminants and additives by chromatography, ultrafiltration or dialysis.

	The buffer used to prepare the antibody solution does not favor complex formation of the antibody with Lipodin-Ab™ reagent.	Change the antibody dilution buffer to PBS, pH7.4. Avoid using Hepes, HBS or Tris buffers. Only use serum-free medium to prepare the complexes.
	Unusual kinetics of delivery due to antibody properties. The delivery efficiency can be monitored from 3 hours until 96 hours post-transfection.	Monitor antibody activity over a longer period of time to identify peak.
	The Lipodin-Ab™ reagent-antibody complexes are aggregated.	The mixture of the two components must be prepared and used within 1 hour.
	Cell density is too low or too high. Cell density should be 50-70% confluent at time of delivery for best results.	Reduce cell density by splitting the cells and wait 24 hours before the delivery experiment. Or, increase cell density by growing cells for one more day before the experiment.
	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.	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.
	Culture medium composition is not optimal for transfection. Although serum was not a factor for the delivery of the antibodies tested, serum-containing media could impair the transfection of certain antibodies for particular cell types.	Use serum-free culture medium during the first 4 hours of transfection.
Cellular toxicity	The antibody delivered is cytotoxic or triggers cell death.	Use suitable controls such as cells alone, Lipodin-Ab™ reagent alone or FITC-labeled IgG positive control provided in the kit.
	Antibody solution contains contaminants or additives that are cytotoxic.	Remove contaminants and additives by chromatography, ultrafiltration or dialysis.
	Cells are not healthy due to contamination by bacteria, mycoplasma or fungus, or due to incorrect cell density or culture media.	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).

4.3. Related Products

Lipodin-Pro™ – The Ultimate Protein Transfection Reagent

Catalog No.	Reagent	Unit
500100	Lipodin-Pro™	25 rxn
500110	Lipodin-Pro™	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
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4.5. Product Use Limitations

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