

Ab-DeliverIN™ - Antibody Delivery Reagent Results

OZ Biosciences is delighted to announce the launching of the innovative **Ab-DeliverIN™ - Antibody Delivery Reagent**. **Ab-DeliverIN™** is a lipid based formulation allowing the delivery of antibodies in living cells. The antibodies delivered inside cells with **Ab-DeliverIN™** retain their structure and function, there is no need to covalent linking, just mix the antibody delivery reagent with your antibody. **Ab-DeliverIN™** reagent forms non-covalent complexes with antibodies. Complexes are internalized by cells and antibodies are released into the cytoplasm without any cytotoxicity.

Principal **Ab-DeliverIN™** advantages:

1. Efficient antibody delivery in a wide variety of cells including primary cells.
2. Suitable for various antibodies (polyclonal, monoclonal)
3. Ready to use reagent
4. High cell viability - No cytotoxicity (biodegradable lipids).
5. Rapid and straightforward procedure
6. Compatible with and without serum-containing media.

Antibody Delivery

Delivery systems allowing exogenous antibodies to be transported inside living cells represent a powerful approach for functional studies or therapeutic approaches. It also opens novel strategies to elucidate new molecular mechanisms. **Ab-DeliverIN™** is a formulation of lipids able to capture antibodies through electrostatic and hydrophobic interactions and deliver them inside cells. **Ab-DeliverIN™ - Antibody Delivery Reagent** delivers antibodies in living cells without biochemical modification and these antibodies remain intact in term of structure and function. The **Ab-DeliverIN™** reagent / antibody non-covalent complexes are fully biodegradable and non cytotoxic. Examples of potential applications are: 1) Intracellular localization studies in living cells, 2) Protein function with blocking antibodies, 3) Protein-protein interaction blocking, 4) FRET studies...

Antibodies Delivered

Several polyclonal & monoclonal antibodies were efficiently delivered in a wide variety of cells with the **Ab-DeliverIN™ - Antibody Delivery Reagent**.

- The antibodies assayed were produced from various species: *human, mouse, rabbit and rat*.
- These antibodies were *polyclonal* IgG from different species and *monoclonal* (anti-Giantin, anti-Nuclear Pore Complex, NPC)
- They were labeled or not with various *fluorophores*:
 - FITC
 - TRITC
 - AlexaFluor®488
 - AlexaFluor®546

It is highly critical that the antibody to be delivered is very pure. It is clear that any impurities, contaminants or additives present with your antibody might affect the delivery efficiency. Indeed, a lot of commercially available antibodies contain additives in large excess such as BSA. Consequently, if BSA is present in your antibody sample, we recommend removing it before proceeding with the delivery assay.

Cell Types Successfully Tested

Ab-DeliverIN™ - Antibody Delivery Reagent is suitable for numerous cell types. This reagent has been successfully tested on a variety of immortalized cell lines as well as some primary cells (see Table 1 page 2). If a particular cell type is not listed, this does not imply that **Ab-DeliverIN™** reagent is not going to work. An updated list of cells effectively tested is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

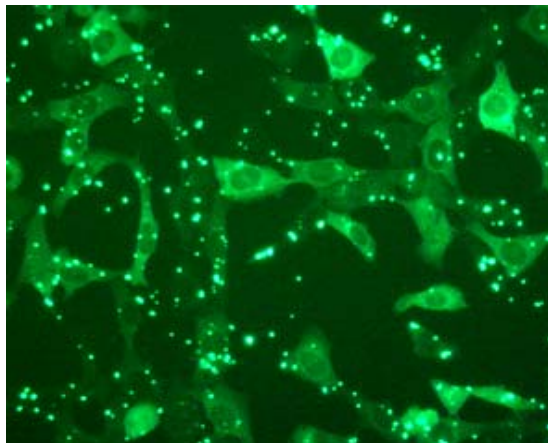
Table 1: Example of cells successfully assayed with **Ab-DeliverIN™ - Antibody Delivery Reagent.**

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

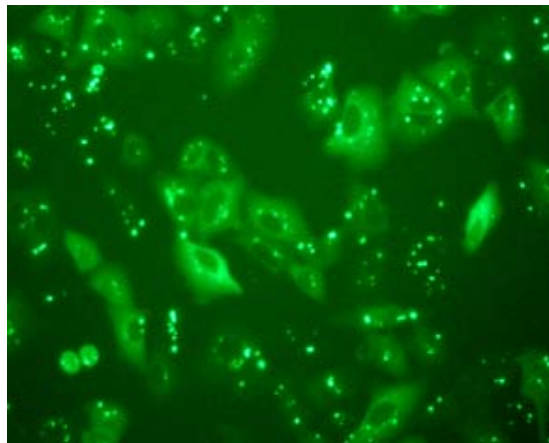
Polyclonal IgG Delivery in Various Cells

1- Fluorescently-labeled IgG delivery.

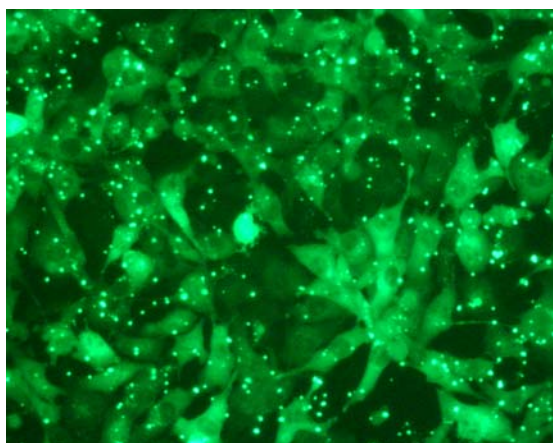
NIH-3T3



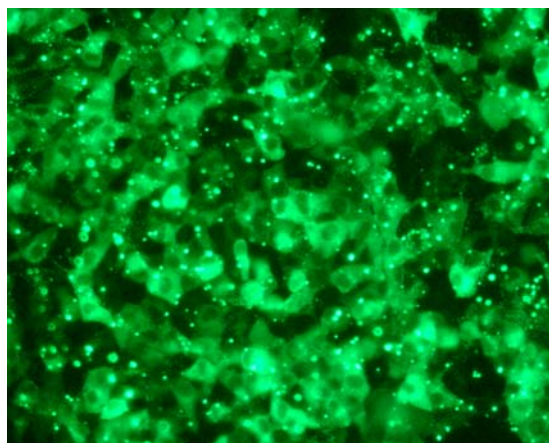
A549



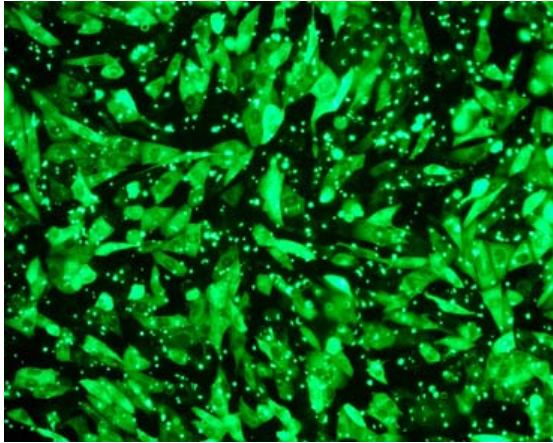
BHK21



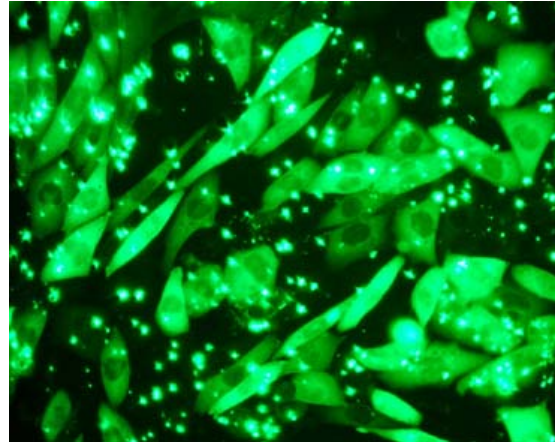
BHK21



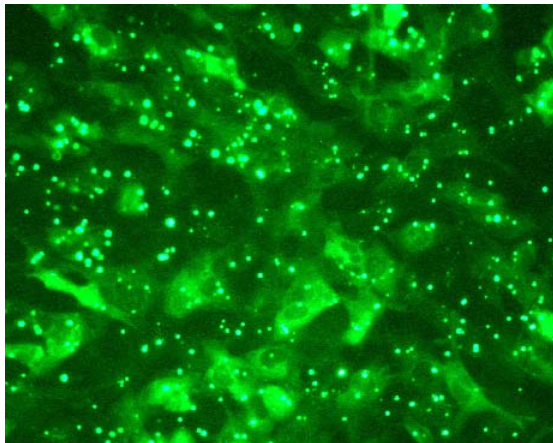
CHO-K1



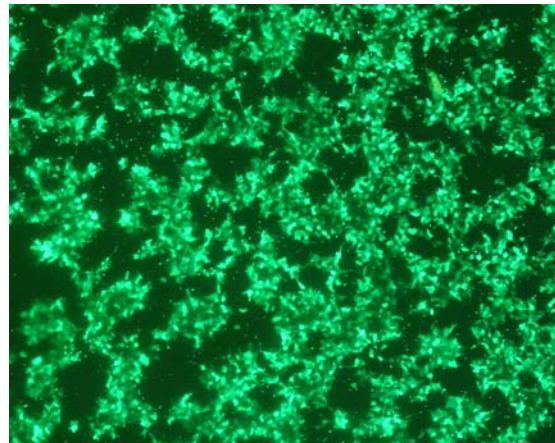
CHO-K1



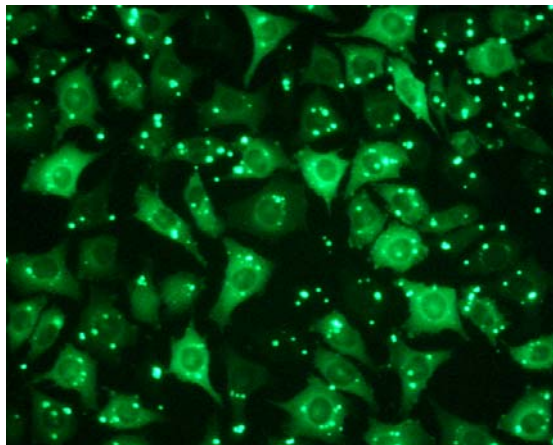
BEAS-2B



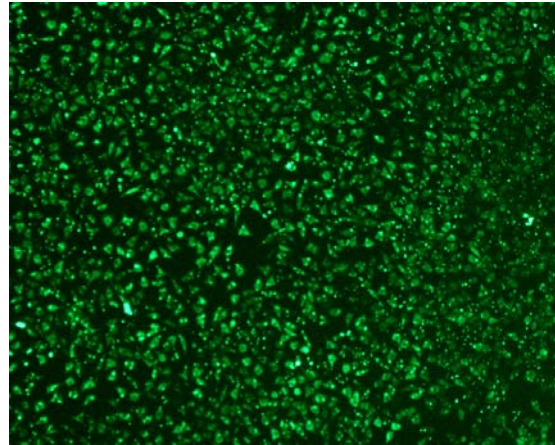
HEK 293



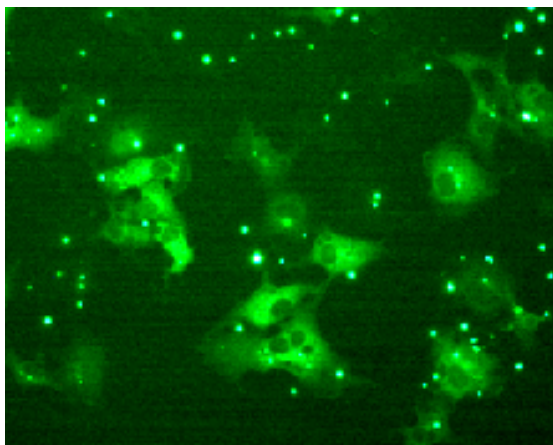
L929



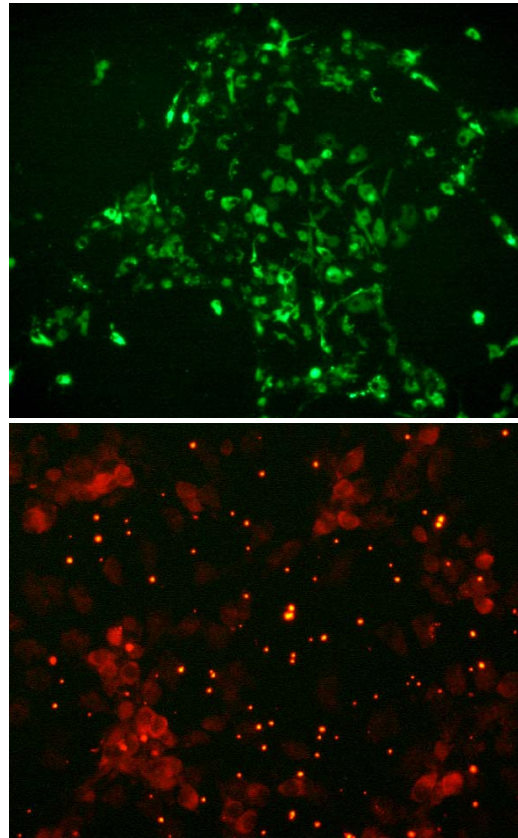
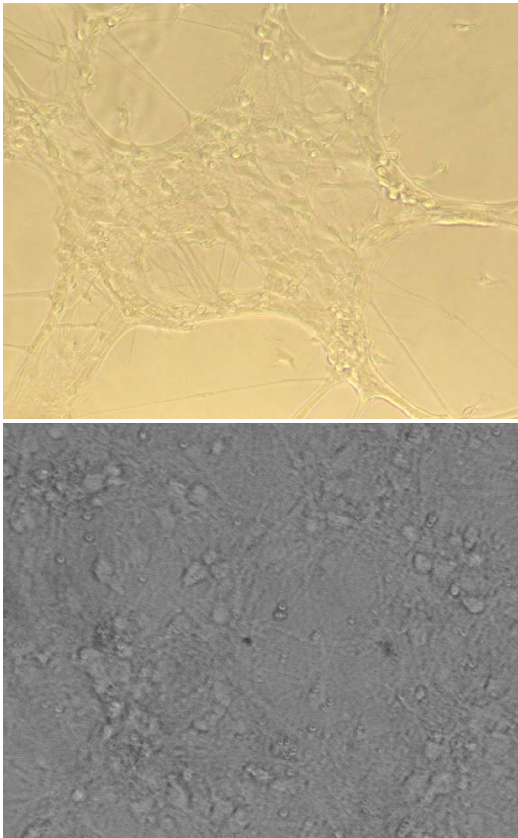
L929



COS 7



Polyclonal IgG from human serum was labeled with FITC (fluorescein isothiocyanate) resulting in 4 FITC molecules bound per IgG molecule. 1 μ g of FITC-IgG was mixed with 2 μ L of **Ab-DeliverIN**[™] reagent and incubated 4 hours with different cell lines in 24-well plates. Living cells or cells fixed with Formalin were then observed by fluorescence microscopy.



0.5 μg of antibody - AlexaFluor®488 labeled or antibody -AlexaFluor®546 labeled were mixed with 4 μL of **Ab-DeliverIN™** reagent and incubated 24 hours with a co-culture of primary neurons and primary glial cells from rat in 35 mm dishes. Cells were observed unfixed by fluorescence microscopy. We are very grateful to L. Efthimiadi and Dr S. Krantic (INMED-Marseille) for their contribution.

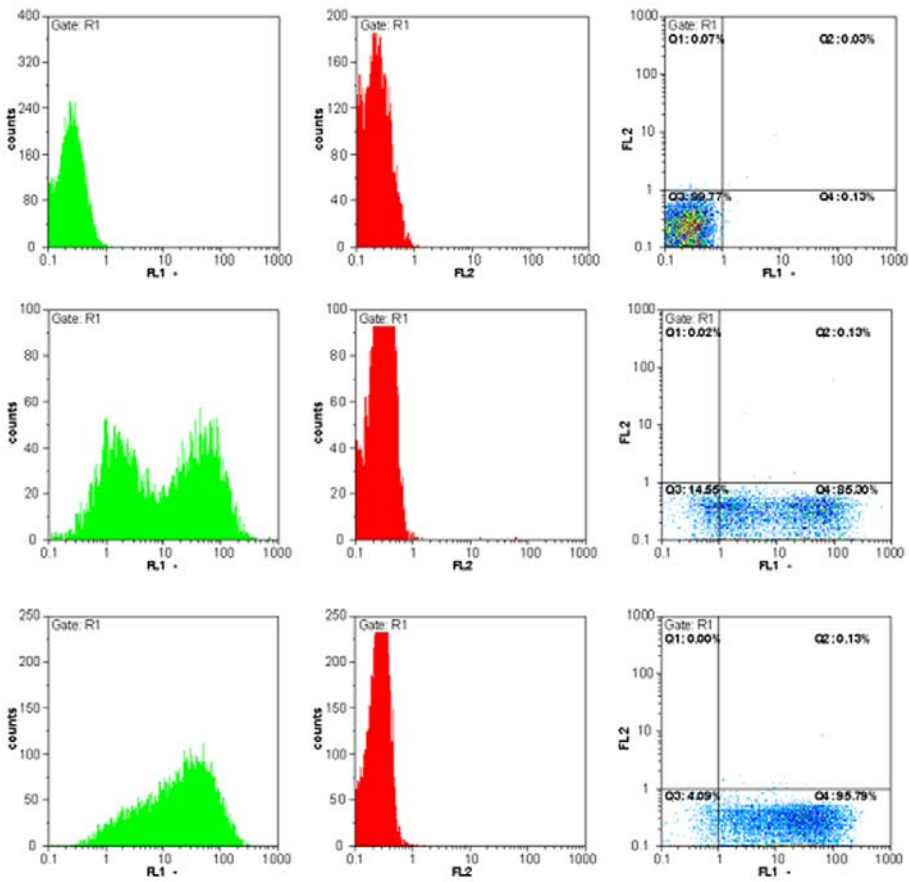


Figure A

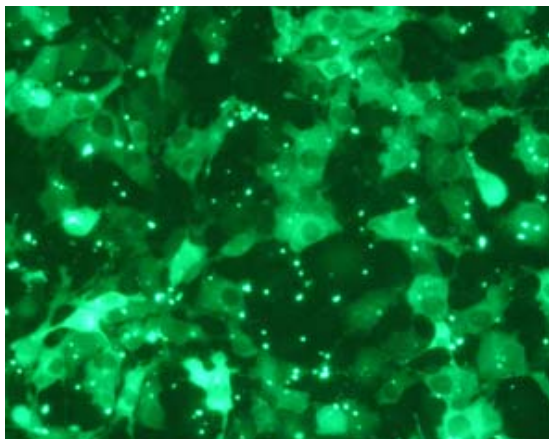
Figure B

Figure C

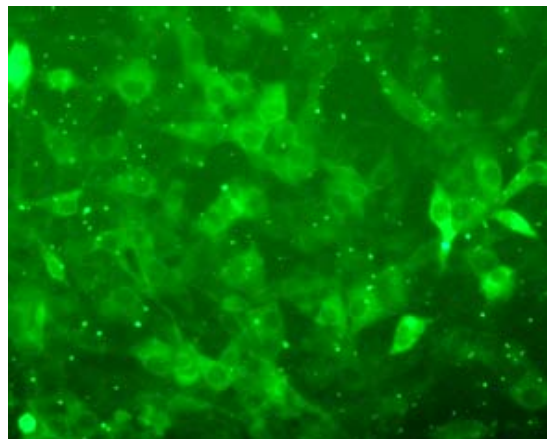
FITC-labeled IgG (5 μg) was mixed with **Ab-DeliverIN™** reagent (10 μL or 5 μL , Figure B and C respectively) and incubated 3 hours with 1×10^6 **U937** cells in a 6-well plate. Cells fluorescence was monitored by cytofluorimetry after 3 h of incubation time. FL1 is standing for FITC fluorescence whereas FL2 indicates propidium iodide fluorescence. Untreated cells are shown in Figure A. FITC-Ab was delivered efficiently in U937 cells and no toxicity was observed. We are very grateful to Ms Bertram (High School of Medicine, Hanover, Germany) for her contribution.

Conclusion: All the antibodies assayed were efficiently delivered in a number of cell types including primary neurons and glial cells. The labeling appears as a diffuse signal in the cytosol. The successful delivery of an antibody was also reached in U937 cells which are very difficult to transfect.

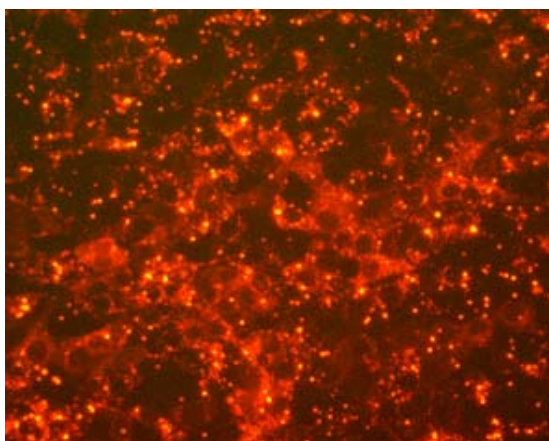
2- Stability of the delivered antibodies.



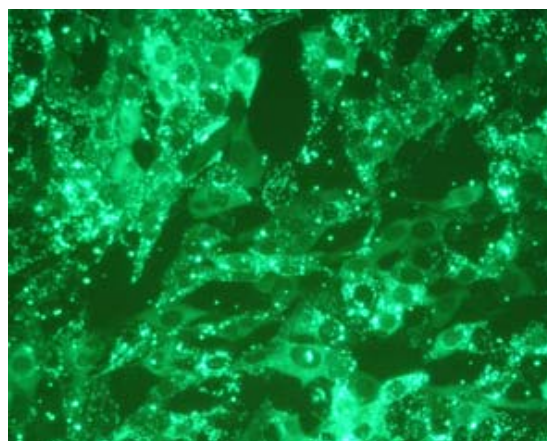
0.5 μg of Ab-AlexaFluor®488 was mixed with 2 μL of **Ab-DeliverIN**[™] reagent and incubated 24h on COS-7 cells in 24-well plates. Cells were then fixed with 2% PFA.



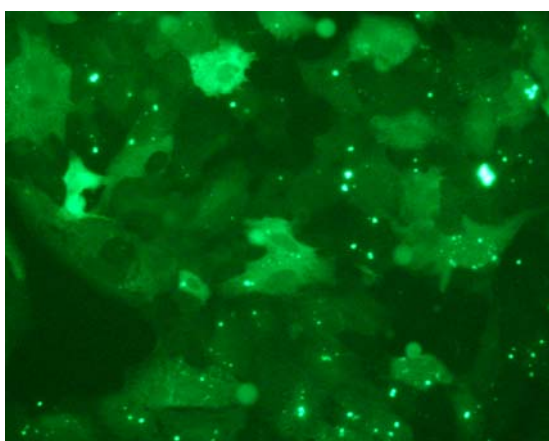
1 μg of Ab-FITC was mixed with 2 μL of **Ab-DeliverIN**[™] reagent and incubated 24 hours on NIH3T3 cells in 24-well plates. Cells were then fixed with 2% PFA.



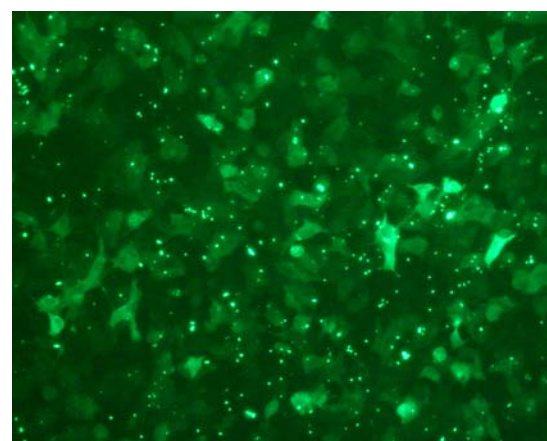
0.5 μg of Ab-AlexaFluor®546 was mixed with 2 μL of **Ab-DeliverIN**[™] reagent and incubated 48 hours on NIH3T3 cells in 24-well plates. Cells were then fixed with 2% PFA.



0.5 μg of Ab-AlexaFluor®488 was mixed with 2 μL of **Ab-DeliverIN**[™] reagent and incubated 48 hours on NIH3T3 cells in 24-well plates. Cells were then fixed with 2% PFA.



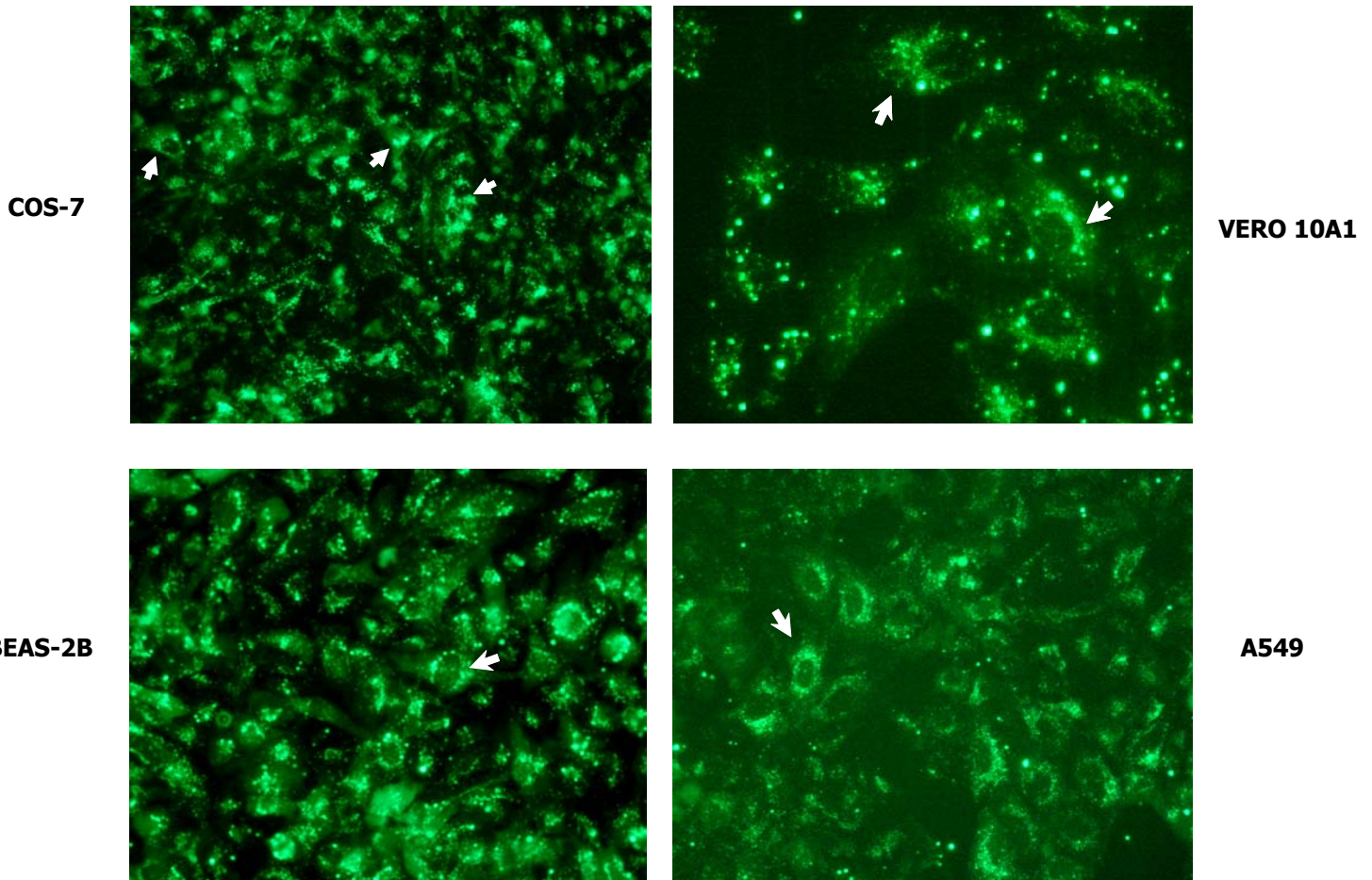
Ab-FITC labeled (1 μg) was mixed with **Ab-DeliverIN**[™] reagent (2 μL) and incubated 72 hours on A549 cells in 24-well plates. Unfixed cells were observed by fluorescence microscopy.



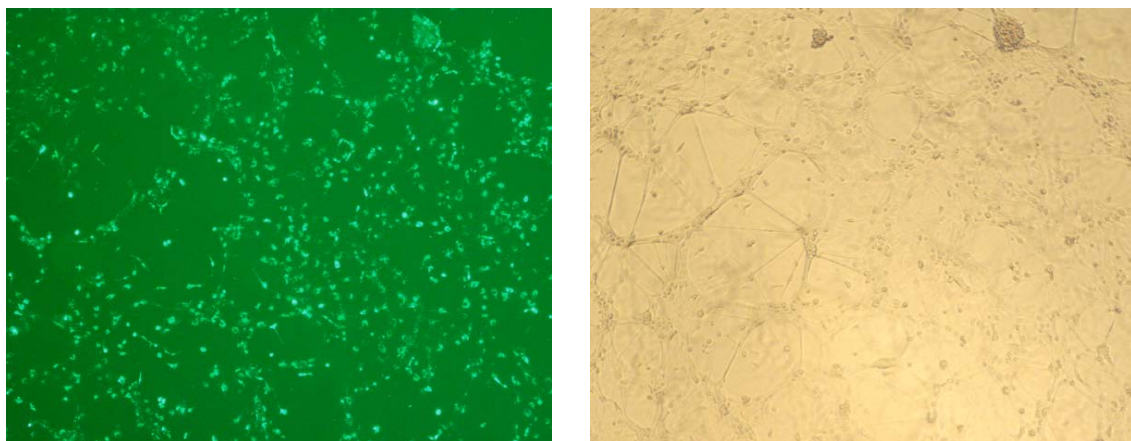
Conclusion: The delivered antibodies can be observed up to several days in the cells. The stability of delivered antibodies depends both on the cell type and on the fluorophore used. Also, highly stable fluorophore allows obtaining good labeling for a longer period of time.

1- Anti-giantin antibody delivery.

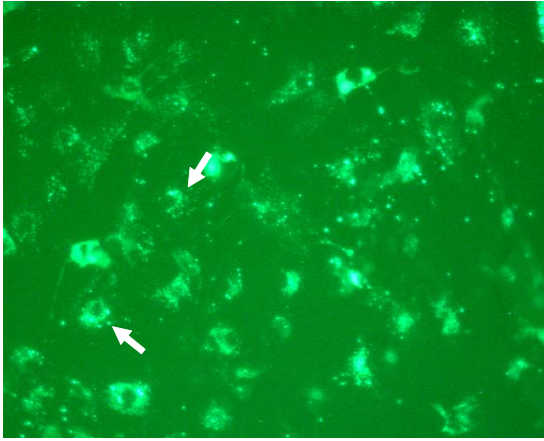
Several polyclonal and monoclonal antibodies were efficiently delivered in various cell lines. In order to check if antibodies can localized properly inside cells upon delivery, anti-giantin antibody was used. Giantin is a Golgi membrane protein containing a large cytoplasmic domain. The antibody used is directed against a cytosolic epitope of the protein and is expected to accumulate in the Golgi area near the nucleus.



0.5 µg of anti-Giantin antibody fluorescently labeled with AlexaFluor®488 was delivered in various cell lines with 2 µL of **Ab-DeliverIN™** reagent in 24-well plates. After 24h of incubation, cells were observed by fluorescence microscopy. White arrows indicate some examples of specific Golgi staining pattern.



Primary neurons and glial cells from rat

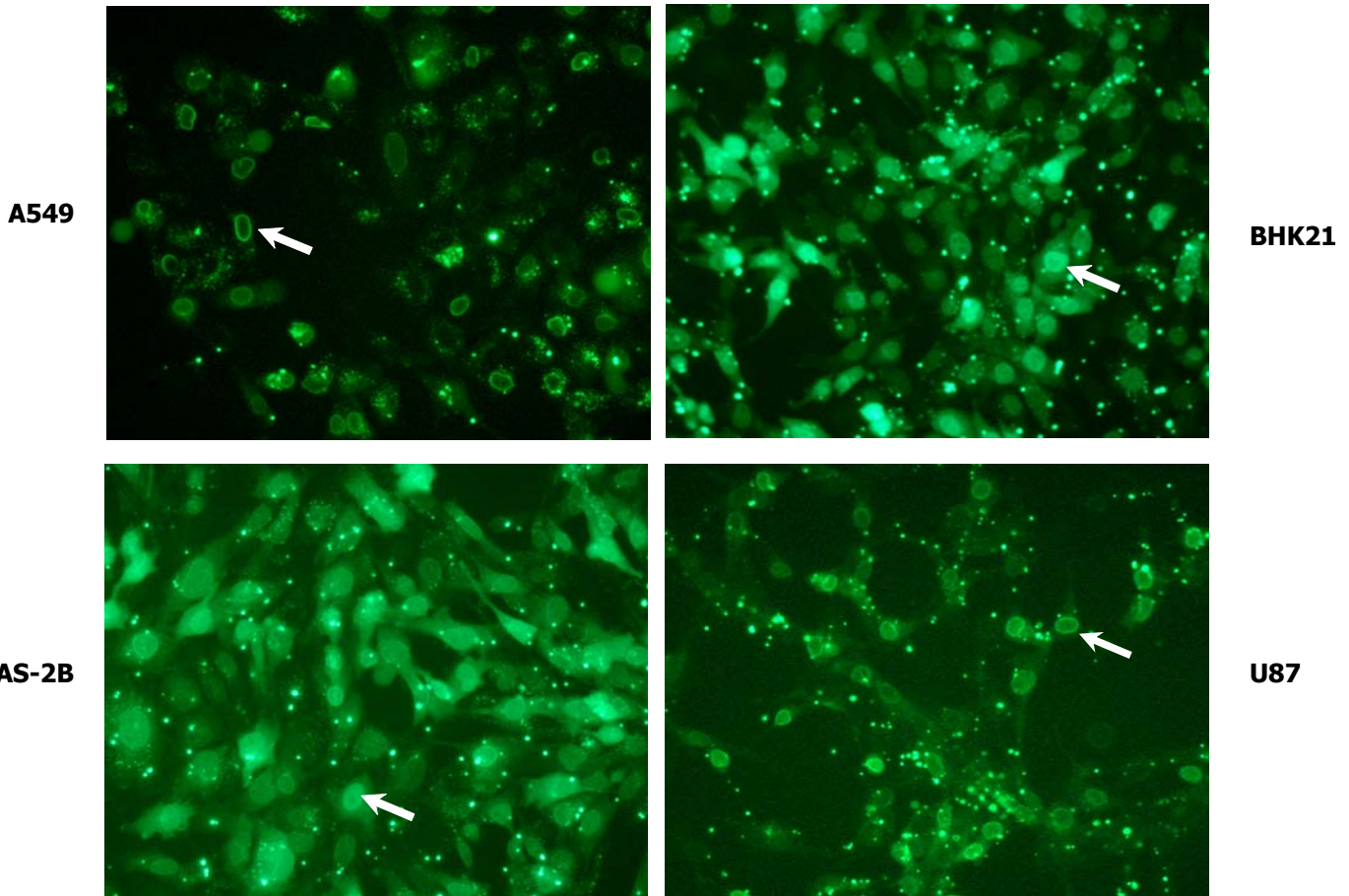


0.5 μg of anti-Giantin antibody fluorescently labeled with AlexaFluor®488 was delivered in primary rat neurons and glial cells with 4 μL of **Ab-DeliverIN™** reagent in 6-well plates. After 72 h of incubation time, unfixed cells were observed by fluorescence microscopy. White arrows indicate some examples of specific Golgi staining pattern

Conclusion: The delivered anti-giantin antibodies accumulate as expected in a region close to the nucleus. It indicates that specific delivery and staining can be performed in live cells with antibodies.

2- Anti-NPC antibody delivery.

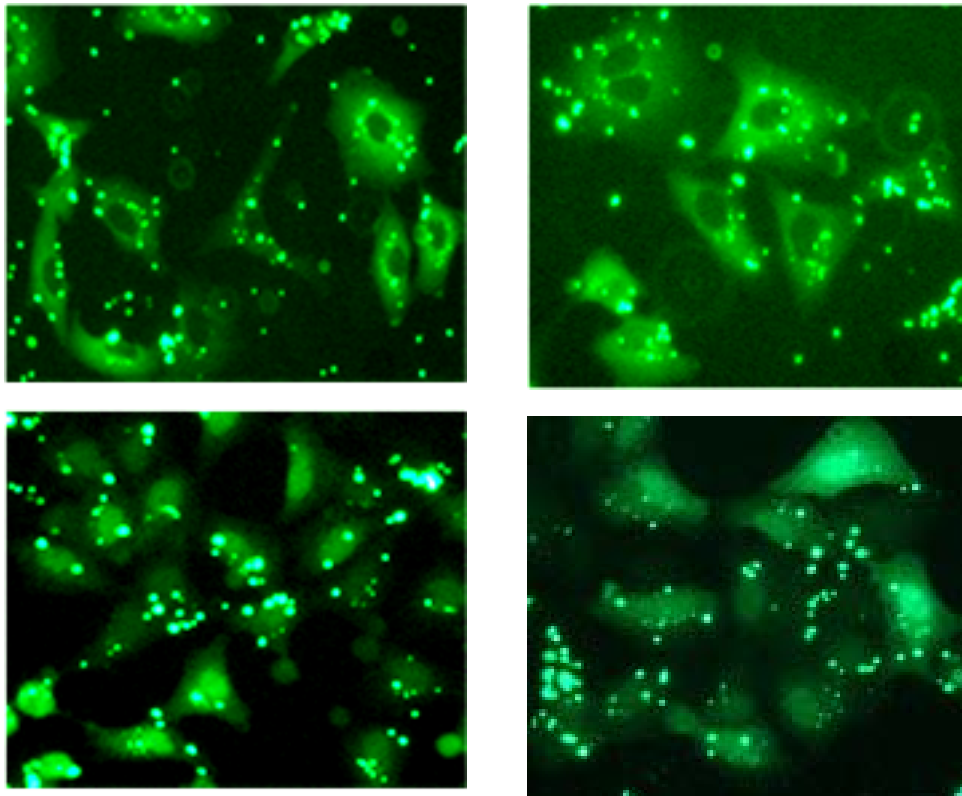
Anti-NPC antibodies are directed against some epitopes of Nuclear Pore Complex proteins. They localize to the nuclear envelope in permeabilized fixed-cells.



0.5 μg of anti-NPC antibody labeled with AlexaFluor®488 was delivered in various cells lines with 2 μL of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. After 6h to 24h of incubation, cells were observed by fluorescence microscopy. White arrows indicate the position of some nuclei.

Conclusion: The delivered anti-NPC antibodies accumulate as expected onto the nuclear envelope. It confirms that intracellular localization of antibodies is not modified upon delivery. Also, it is noticeable that a part of the antibodies are translocated inside the nucleus in live cells. That means that some differences can appear compare to fixed cell labeled by antibodies.

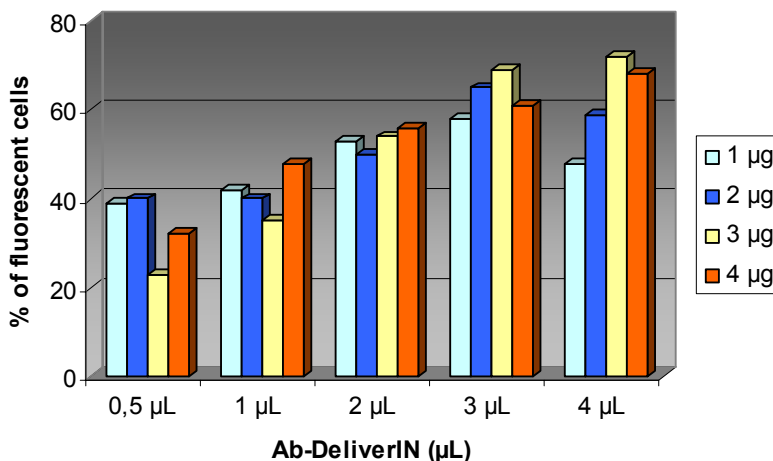
The karyophilic nuclear localization signal (NLS) peptide derived from SV40 was covalently linked to FITC labeled IgG in order to deliver them inside the nucleus.



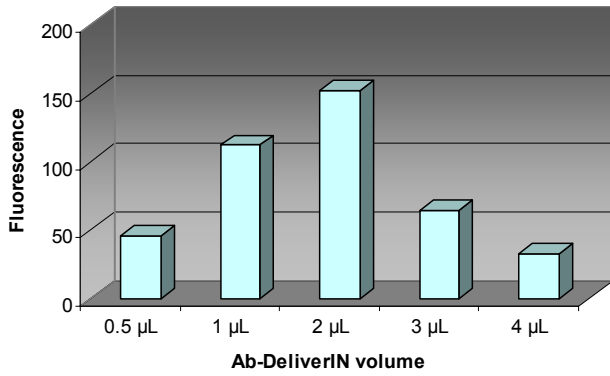
FITC-labeled IgG (1 µg) were delivered in A549 cells with 2 µL of **Ab-DeliverIN™** reagent in 24-well plates. After 16h of incubation, cells were observed by fluorescence microscopy. Top pictures represent fluorescent IgG and bottom pictures represent fluorescent IgG-NLS.

Conclusion: As shown above, the **Ab-DeliverIN™** reagent does not alter antibodies biodistribution upon delivery. The NLS-bearing IgG accumulates as expected in the nucleus in contrast to the IgG.

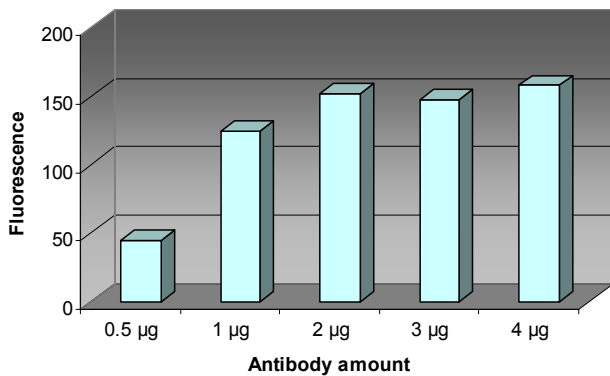
1- Dose-Response studies of antibody Delivery.



The indicated amount of FITC-labeled antibody was delivered in A549 cells with the indicated amount of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. After 4h of incubation time at 37°C, cells were trypsinized and the number of fluorescent cells was determined by cytofluorimetry

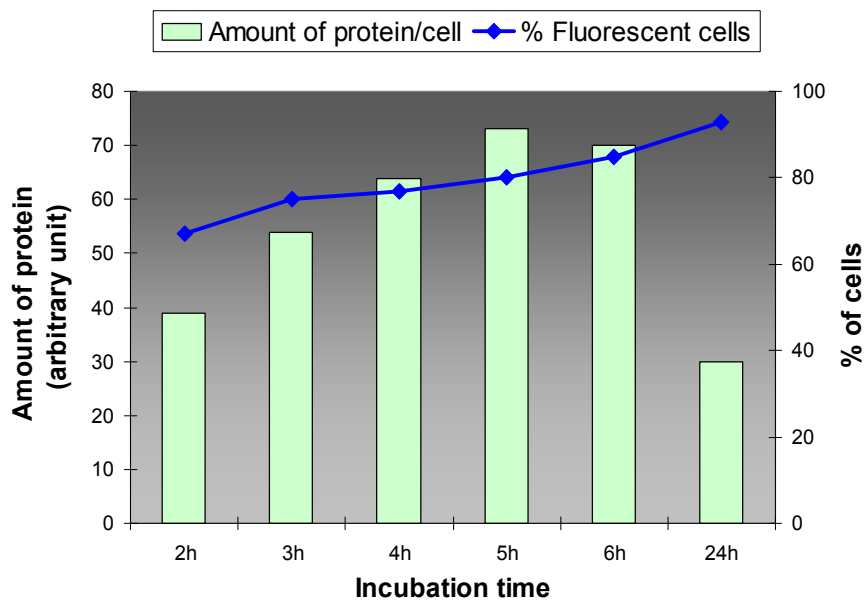


One µg of FITC-labeled antibody was delivered in NIH3T3 cells with the indicated amount of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. Cells were collected after 4h of incubation time and the fluorescence level was monitored by spectrofluorimetry.



The indicated amounts of FITC-labeled antibody were delivered in NIH3T3 cells with 2 µL of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. Cells were collected after 4h of incubation time and the fluorescence level was monitored by spectrofluorimetry.

2- Kinetic of antibody delivery in NIH3T3 cells.



One µg of FITC-labeled antibody was delivered in NIH3T3 cells with 2 µL of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. Cells were collected and fixed with 2% PFA at the indicated time point. The number of fluorescent cells and the mean fluorescence were determined by cytofluorimetry. The mean fluorescence was used to evaluate the amount of antibody internalized inside cells.

3- Amount of antibody delivered.

Presence of FBS during incubation	No	No	Yes	Yes
Treatment with trypsin	Yes	No	Yes	No
% of IgG-FITC recovered in NIH-3T3 cells	18	44	21	32

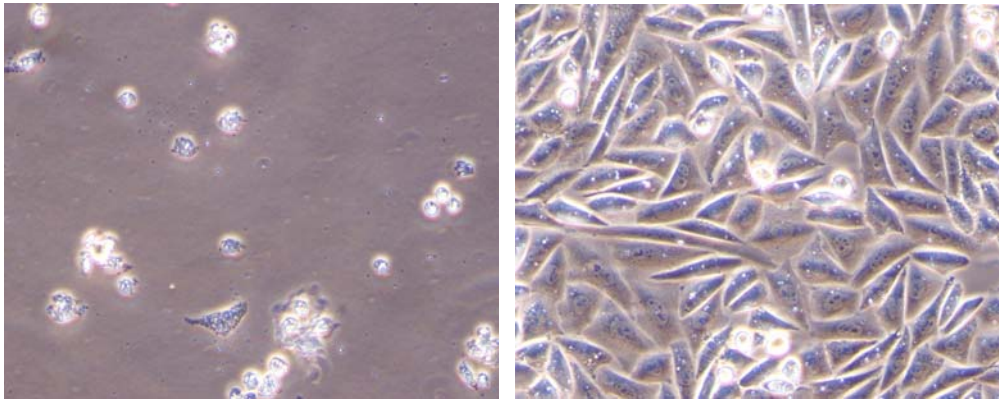
Two micrograms of FITC-labeled antibody were delivered in NIH3T3 cells with 3 μ L of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. The incubation was performed in the presence or in the absence of FBS in the culture medium. After 4h of incubation, cells were trypsinized or not to discriminate the internalized antibody from the complexes adsorbed onto the cell membrane. Finally, cells were lysed, membrane residues were removed by centrifugation and the fluorescence was measured by spectrofluorimetry. The amount of antibodies associated with cell membranes ranged from 30 to 40% depending on the presence of serum or not. However, the results show that 18 % and 21 % of the input material was effectively internalized in the absence or presence of FBS, respectively.

Cells	% of cytosolic antibody-FITC internalized
COS 7	21
HeLa	18
NIH-3T3	21
Vero	14

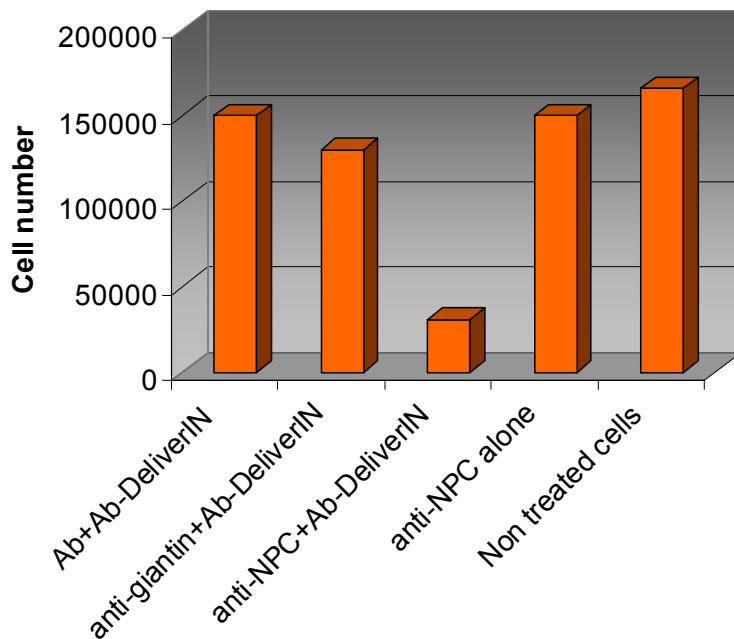
4- Antibody delivery efficiency in various cell lines.

Cell Lines	% of positive cells
3T6	> 50 %
A549	60-80 %
B16-F10	> 50 %
BEAS-2B	80 %
BHK21	80-95 %
CHO-K1	70-95 %
COS-7, COS-1	70-95 %
HEK293	80-100 %
HeLa	60-75 %
L929	80-90 %
NIH3T3	50-75 %
Raw 264.7	90 %
U87	75-95 %
U937	80-90 %
Vero	75-95 %
K562	25-50 %
HaCaT	25-50 %
MDCK	25-50 %
Primary Cells	% of positive cells
Neurons and glial cells	> 50 %

Anti-NPC Antibody Delivery and Biological Activity

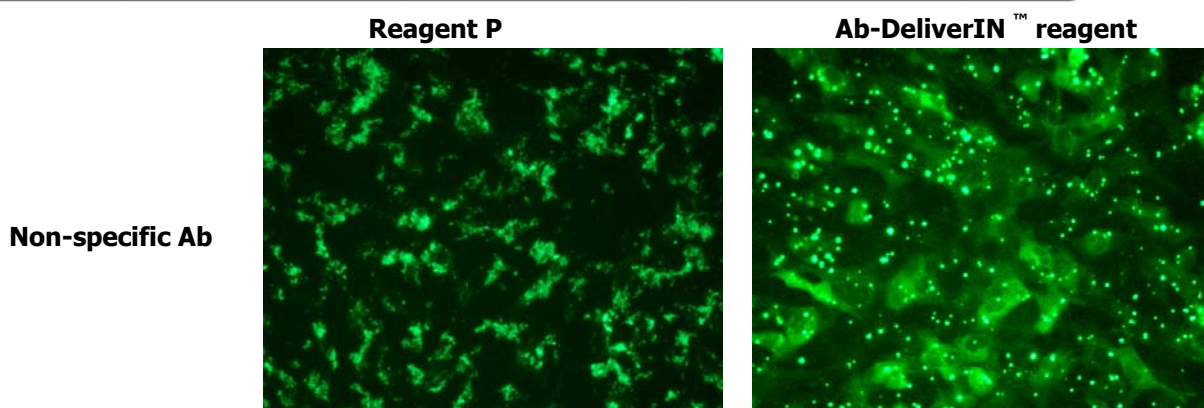


CHO-K1 cells were incubated with 0.5 µg of anti-NPC antibodies and 2 µL of **Ab-DeliverIN™** reagent in 24 well plates (left picture) during 72 hours. As a control, cells were incubated with **Ab-DeliverIN™** reagent alone (not shown) or with **Ab-DeliverIN™** reagent plus a non-specific antibody (right picture). Quantitative data, indicated the number of cells remaining in each well, are presented on the left.

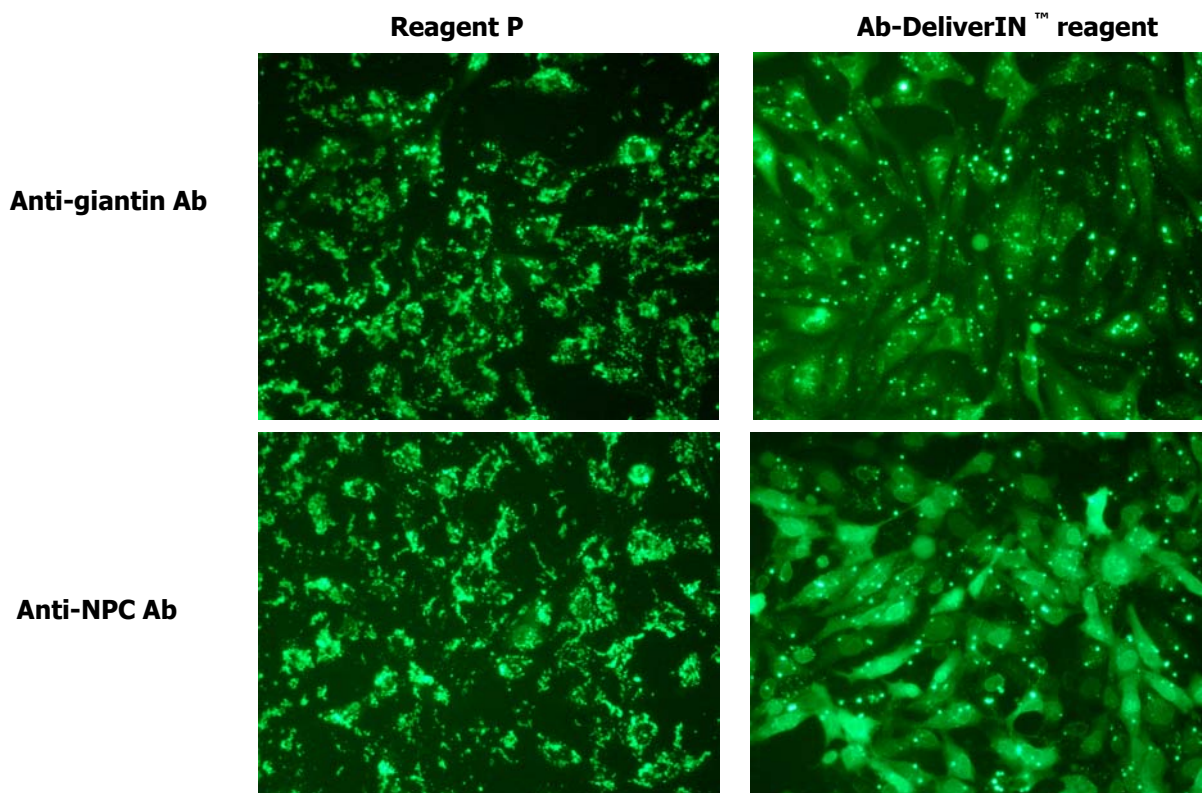


Conclusion: The delivery of the anti-NPC antibody with Ab-DeliverIN™ reagent induces cell death. Although the mechanism of this cell death is unknown, cell division is required to obtain such results. It is likely that the anti-NPC antibody interferes with the nuclear envelope reconstitution after cell division as well as with transport of various molecules from nucleus to cytoplasm and inversely. However we can conclude from such experiments that the delivered antibodies are active and such approaches will allow to assess antibody functions or to study molecular mechanisms.

Comparison with another reagent

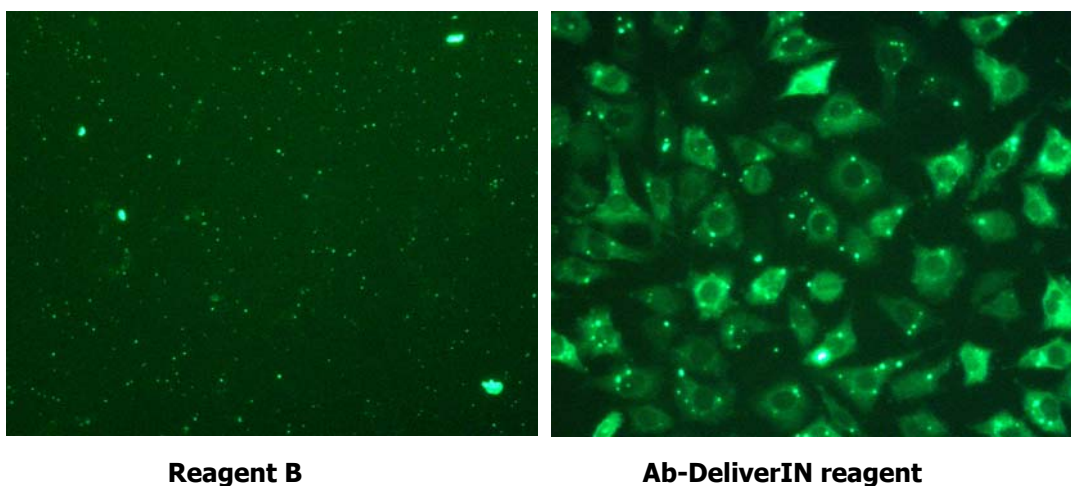


Non-specific Ab



Non-specific antibody (1 µg), anti-giantin antibody (0.5 µg) and anti-NPC antibody (0.5 µg) were delivered in BEAS-2B cells with a competitor **reagent P** (left) or with the **Ab-DeliverIN™ reagent** (right).

Comparison with a competitor in presence of serum



FITC-labeled antibody (1 µg) was delivered in L929 cells with a competitor **reagent B** as described in the manufacturer instruction manual or with the **Ab-DeliverIN™ reagent** in the presence of serum during the 4 h of incubation time. The presence of serum completely inhibited the delivery of antibodies inside cells with the **reagent B** whereas the delivery is very efficient with the **Ab-DeliverIN™ reagent**.