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 both the encapsulation of antibodies in liposomes and their delivery in the cytosol of living cells.

Main features are:

1. Efficient antibody delivery in a wide variety of cells.
2. Various antibodies were delivered in the cytoplasm.
3. Ready to use reagent.
4. Compatible with and without serum-containing media.
5. High cell viability - No cytotoxicity (biodegradable lipids).
6. Rapid and Straightforward procedure.

Antibody Delivery

Ab-DeliverIN™ - Antibody Delivery Reagent allows delivering antibodies, which retain their structure and function, in living cells without the need of fixation. The delivery of antibodies inside living cells represents a powerful approach for functional studies or therapeutic approaches. In order to complete genomic studies or assess the function of a given protein, this innovative approach can provide the scientist with complementary data. For example, delivery of blocking antibodies can complete studies realized with siRNA experiments. **Ab-DeliverIN™** is a lipid based formulation which form non-covalent complexes with antibodies. Complexes are internalized by cells and are released into the cytoplasm without any cytotoxicity.

Cell Types Successfully Tested

Ab-DeliverIN™ - Antibody Delivery Reagent is applicable on numerous cell types. This reagent has been tested on several cell lines and OZ Biosciences is maintaining an updated list of cells successfully tested that is available on the website: www.ozbiosciences.com. If a particular cell type is not listed, this does not imply that **Ab-DeliverIN™ - Antibody Delivery Reagent** is not going to work.

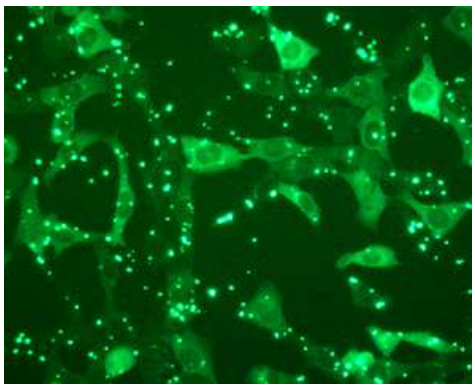
Cell Line	***Cell Type***	***Source***
3T6	Embryonic fibroblasts	Mouse
A549	Non-small cell lung carcinoma	Human
B16-F10	Melanoma	Mouse
BEAS-2B	Bronchial epithelial cells	Human
BHK21	Fibroblasts (Kidney)	Hamster
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblast (Kidney)	Green Monkey
HaCaT	Keratinocytes	Human
HEK-293	Transformed Embryonic (Kidney)	Human
HeLa	Cervical Epithelial Carcinoma	Human
Jurkat	T cell leukemia	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		**Source**
Primary neurons		Rat
Primary glial cells		Rat

Antibodies Delivered

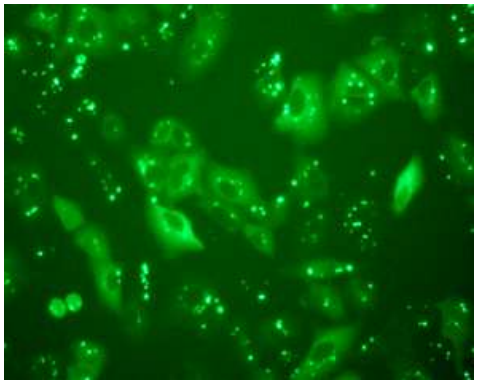
Several antibodies were efficiently delivered in living cells with the **Ab-DeliverIN™ - Antibody Delivery Reagent.** These antibodies are human, mouse and goat IgG-FITC, Human IgG-TRITC, rabbit anti-Giantin IgG AlexaFluor®488, mouse anti-NPC IgG AlexaFluor®488, mouse IgG AlexaFluor®488, mouse IgG AlexaFluor®546.

Antibodies Delivery in Various Cells

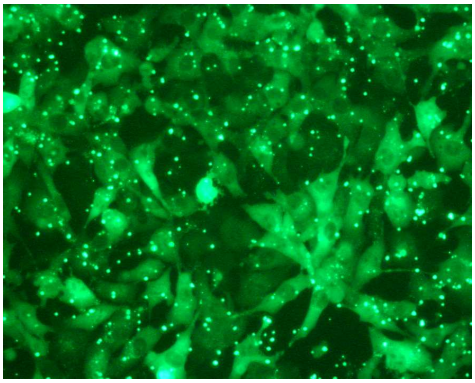
1- Fluorescent antibody delivery.



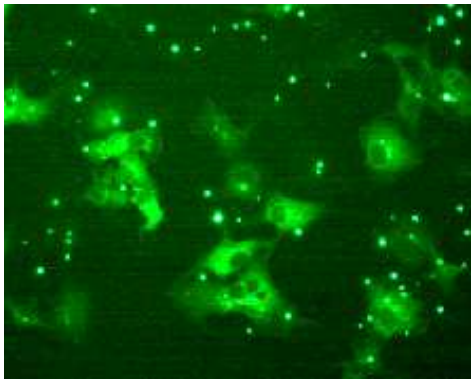
NIH3T3



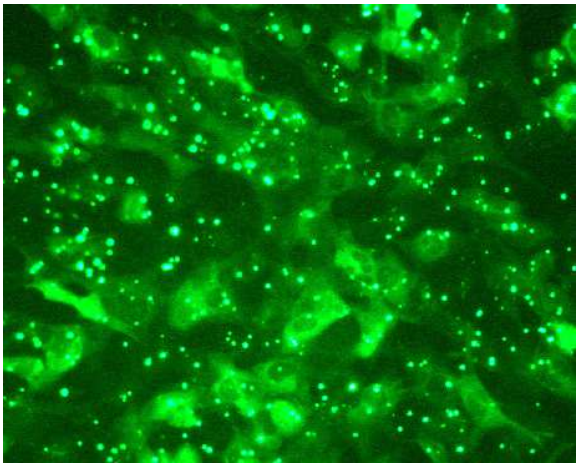
A549



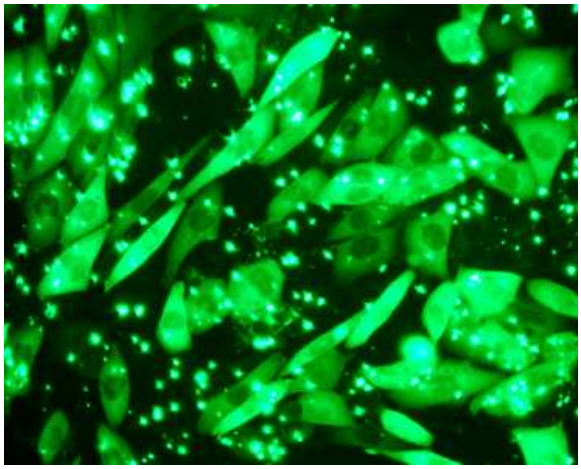
BHK21



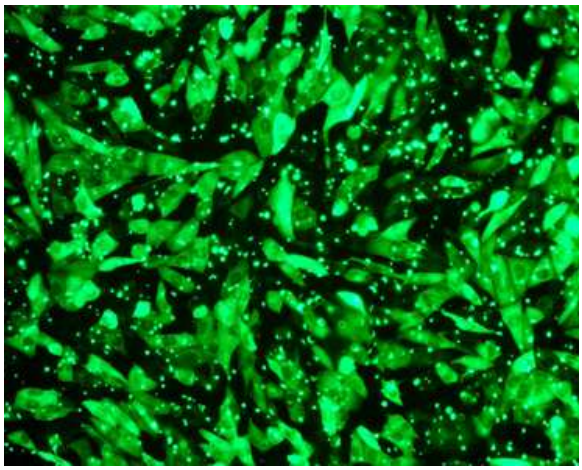
COS-7



BEAS-2B



CHO



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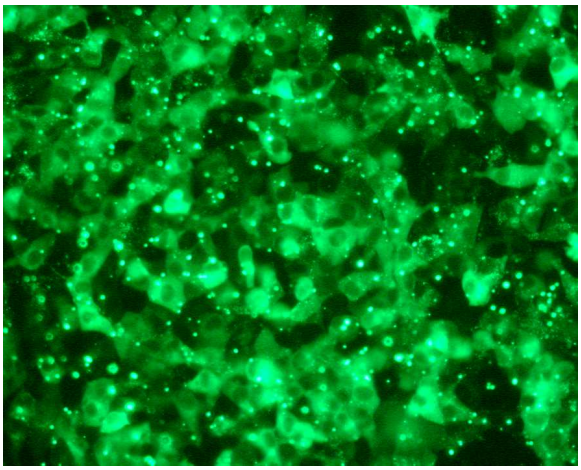

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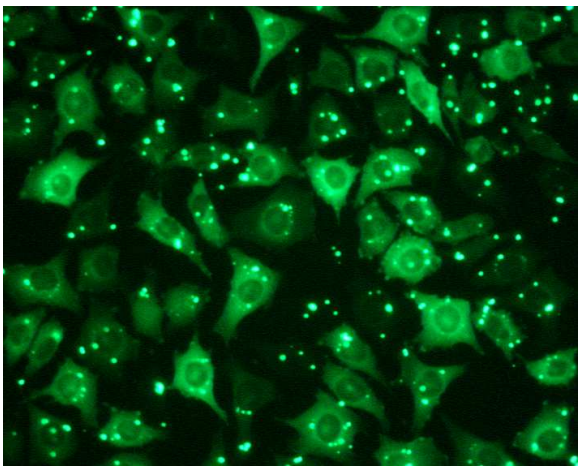
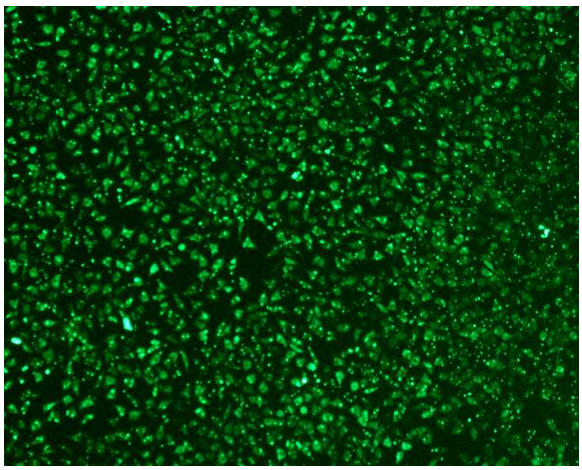
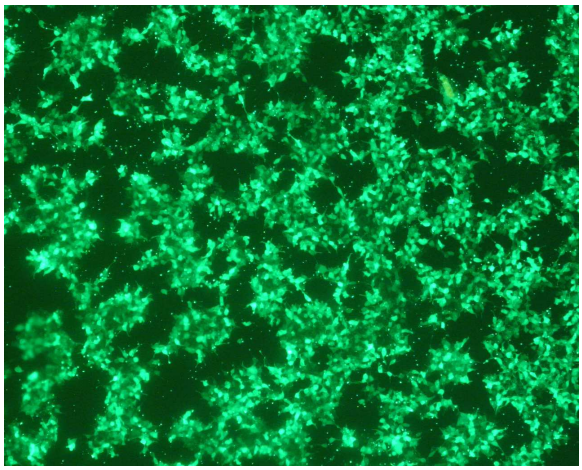


L929

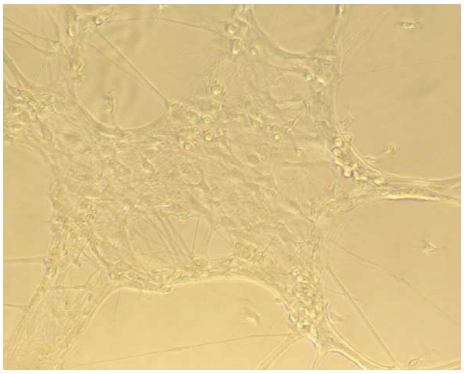


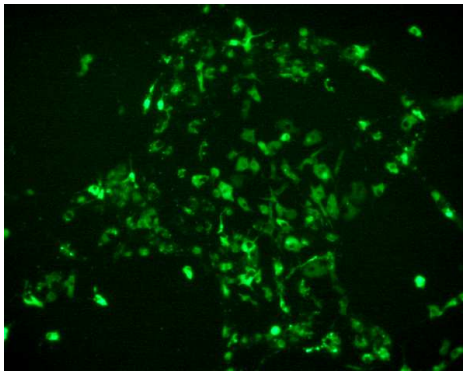
L929

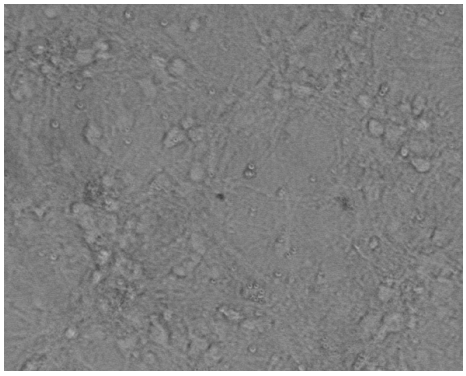


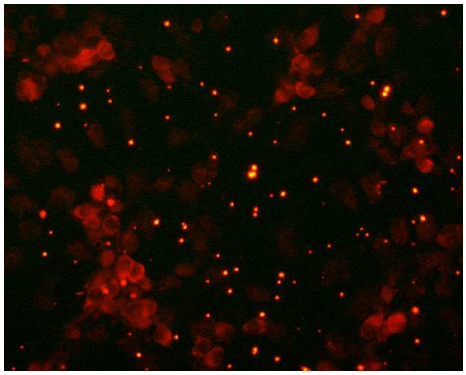
HEK 293

IgG from human serum (Sigma-Aldrich) was labeled with FITC ending with 4 fluorescein molecules bound per IgG molecule. Ab-FITC labeled (1µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 4 hours with the above cell lines in 24-well plates. Cells were observed by fluorescence microscopy either before or after fixation with Formalin.









Ab-AlexaFluor®488 labeled (0.5 µg) or Ab-AlexaFluor®546 labeled (0.5 µg) from Molecular Probes were mixed with **Ab-DeliverIN™** (4 µL) 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.

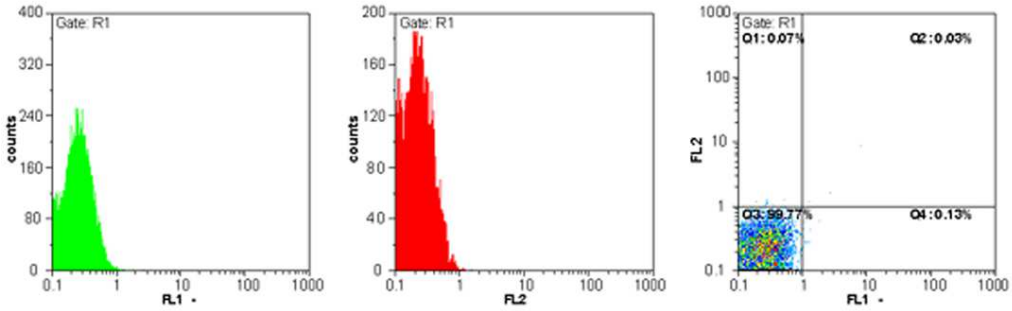


Figure A

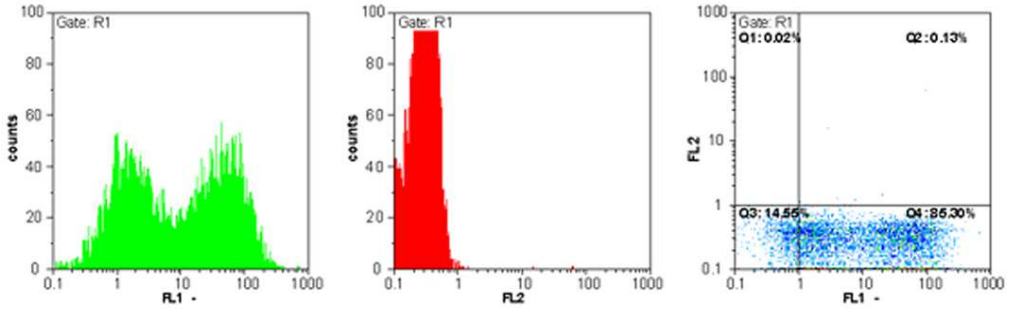


Figure B

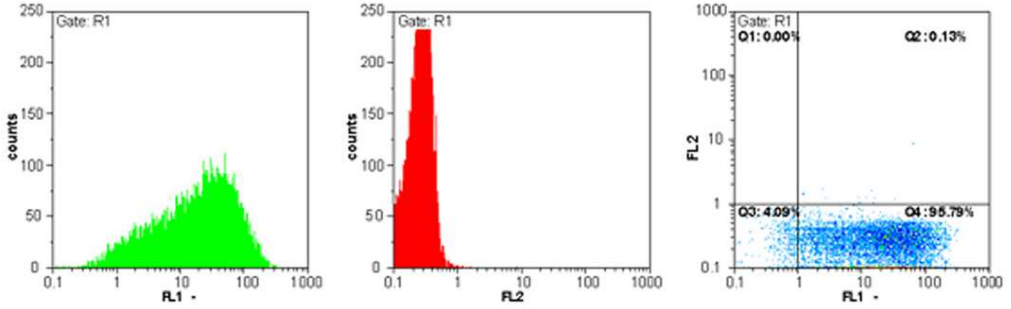
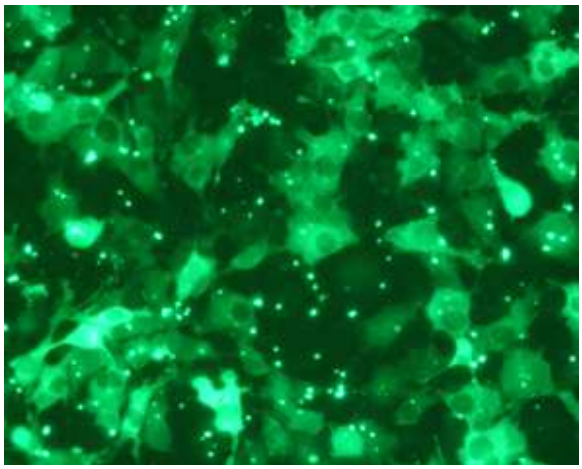


Figure C

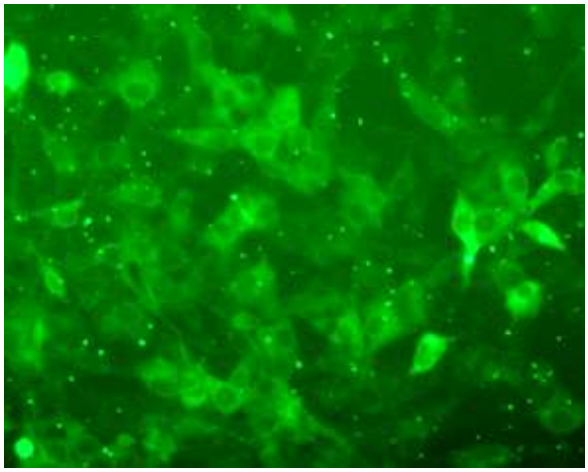
FITC-labeled IgG (5 µg) was mixed with **Ab-DeliverIN™** (10 µL or 5 µL, Figure B and C respectively) and incubated 3 hours with $1x10^6$ U937 cells in a 6-well plate. Cells fluorescence was monitored by cytofluorimetry after 3 h of incubation time. FL1 indicates FITC fluorescence whereas FL2 indicates 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 (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.

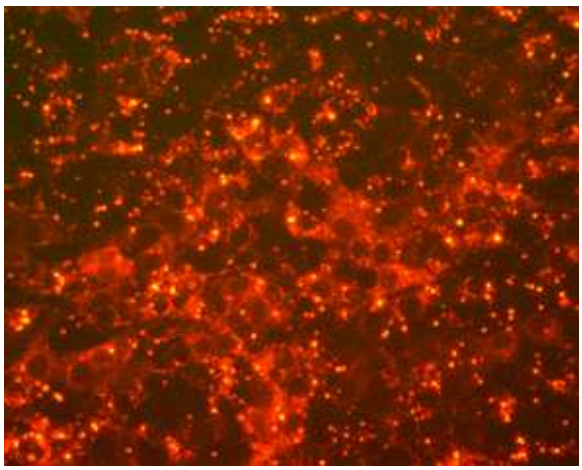
2- Stability of the delivered antibodies.



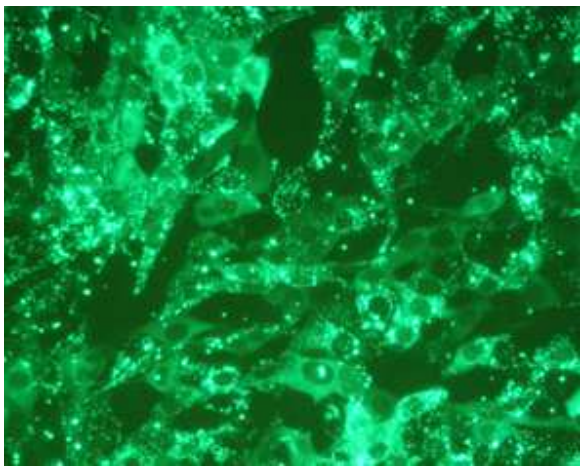
Ab-AlexaFluor®488 labeled (0.5 µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 24 hours with COS-7 in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.



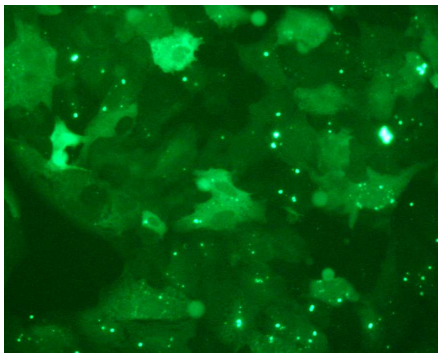
Ab-FITC labeled (1 µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 24 hours with NIH3T3 in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.

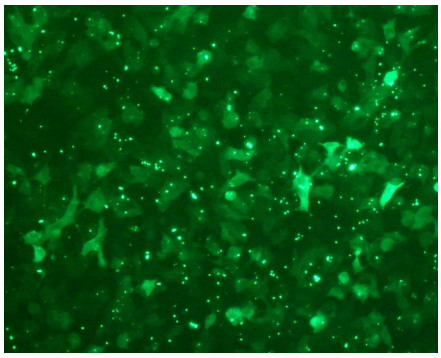


Ab-AlexaFluor®546 labeled (0.5 µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 48 hours with NIH3T3 in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.



Ab-AlexaFluor®488 labeled (0.5 µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 48 hours with NIH3T3 in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.





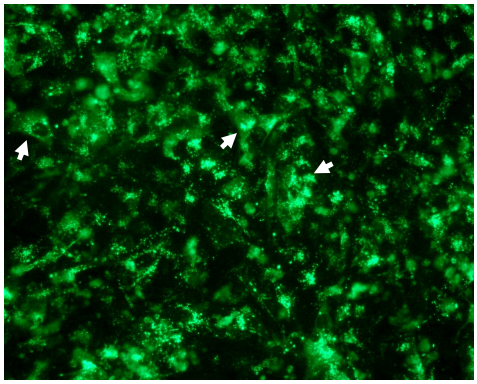
Ab-FITC labeled (1 µg) was mixed with **Ab-DeliverIN™** (2 µL) and incubated 72 hours with A549 in 24-well plates. Unfixed cells were observed by fluorescence microscopy.

Conclusion: The delivered antibody 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.

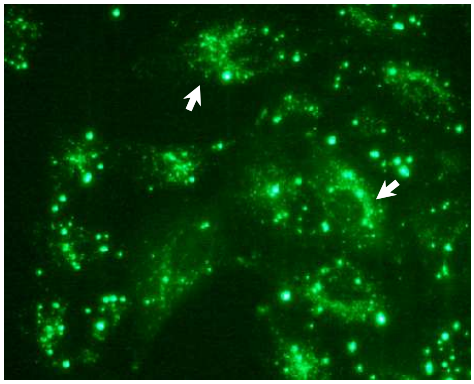
Intracellular Localization of Delivered Antibodies

1- Anti-giantin antibody delivery.

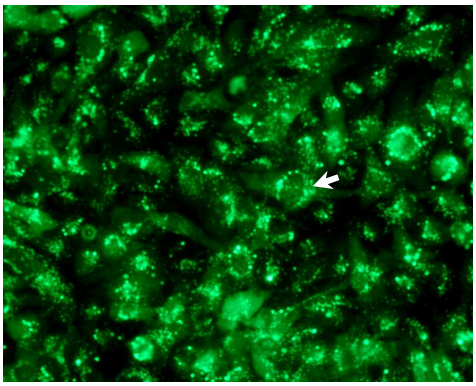
Several different antibodies were efficiently delivered in various cell lines. In order to check if antibodies can localized properly inside cells upon delivery, anti-giantin antibody was used. Giantin is a Golgi membrane antibody containing a large cytoplasmic domain. The antibody used is directed against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



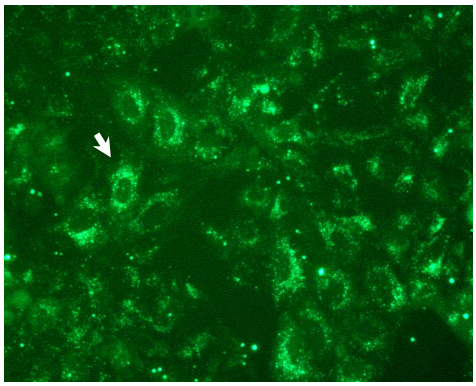
COS-7



VERO 10A1

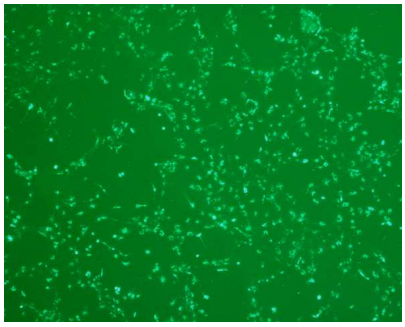


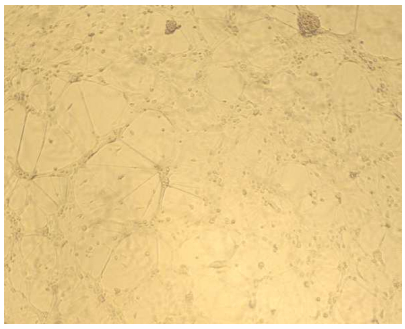
BEAS-2B

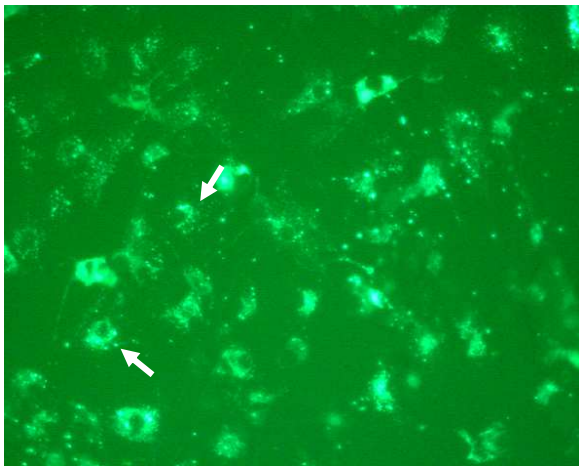


A549

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





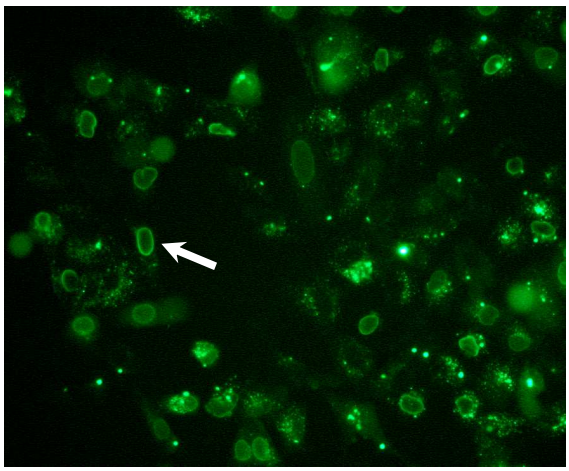


0.5 µg of anti-Giantin antibody AlexaFluor®488 labeled (Eurogentec) was delivered in primary rat neurons and glial cells with 4 µL of **Ab-DeliverIN™** in 6-well plates. After 72 h incubation time unfixed cell were observed by fluorescence microscopy. White arrows indicate some examples of specific Golgi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

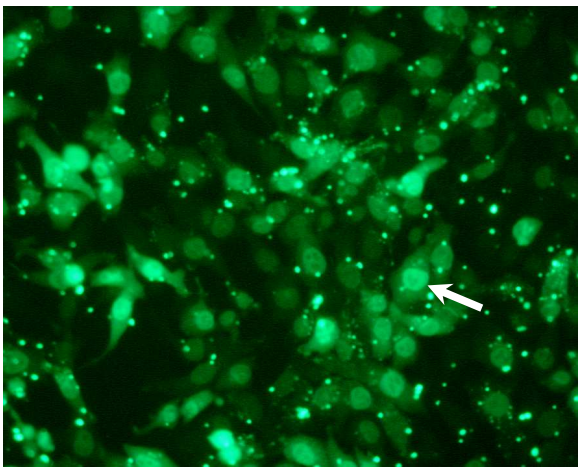
Conclusion: The delivered anti-giantin antibodies accumulate as expected in a region close to the nucleus. It indicates that specific delivery and staining can be performed in live cells with antibodies.

2- Anti-NPC antibody delivery.

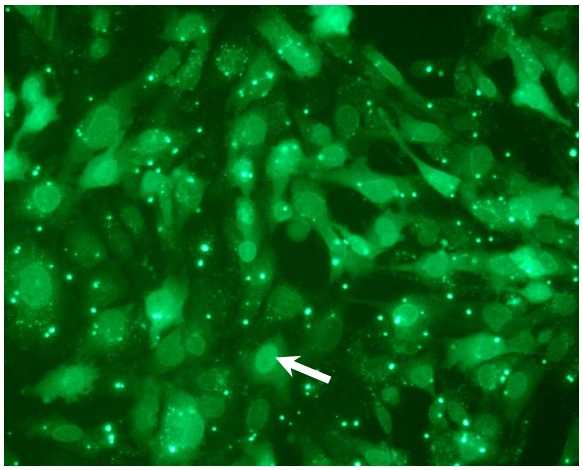
Anti-NPC antibodies are directed against some epitopes of Nuclear Pore Complex antibodies. They localize to the nuclear envelope in permeabilized fixed-cells.



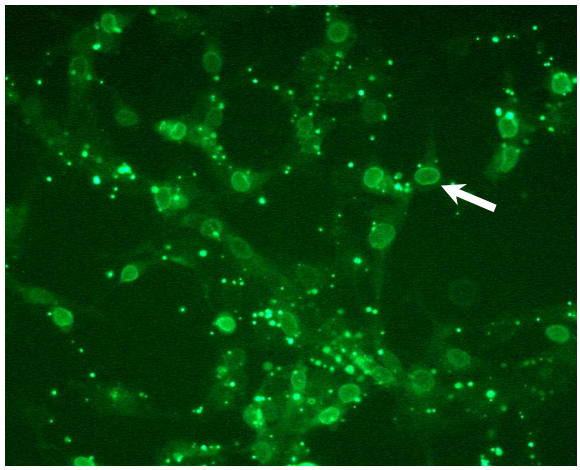
A549



BHK21



BEAS-2B



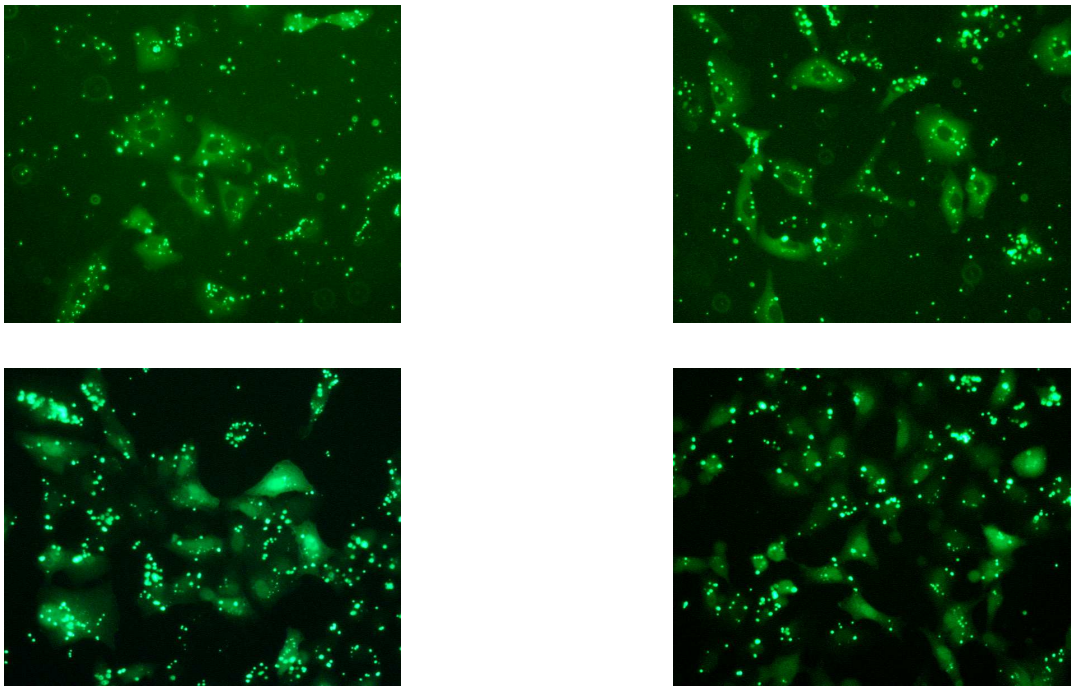
U87

0.5 µg of anti-NPC antibody AlexaFluor®488 labeled (Abcam) was delivered in various cells with 2 µL of **Ab-DeliverIN™ Antibody Delivery Reagent** in 24-well plates. After 6h to 24h incubation time cell 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 expected 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 staining by antibodies.

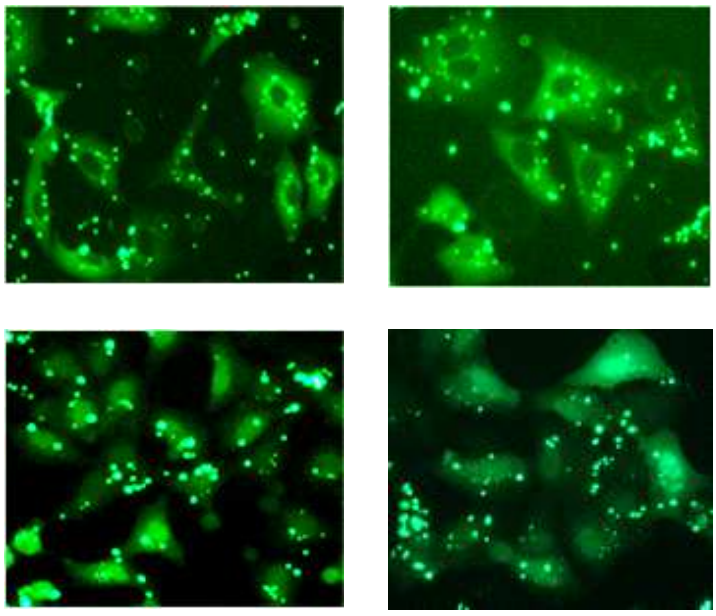
NLS linked antibodies Delivery

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



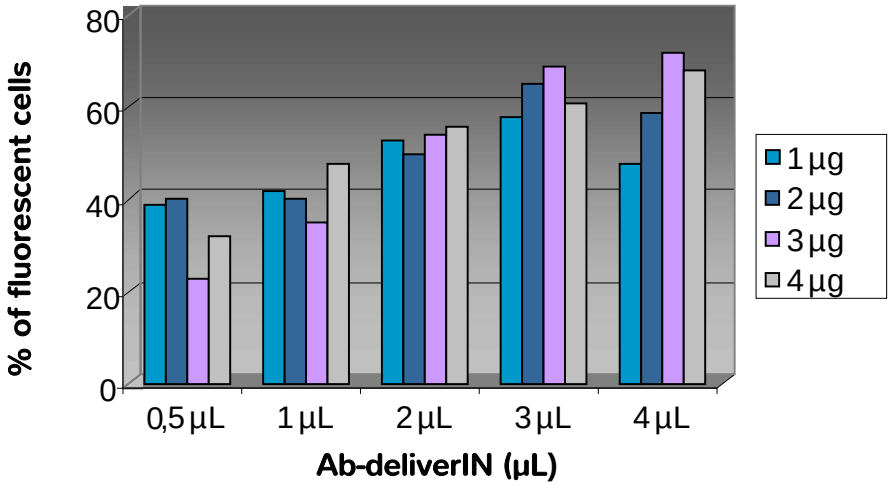
FITC-labeled IgG (1 µg) were delivered in A549 cells with 2 µL of **Ab-DeliverIN™ - Antibody Delivery Reagent** in 24-well plates. After 16h incubation time cell were observed by fluorescence microscopy. Top pictures represent IgG and bottom pictures represent NLS-bearing Ab.

Conclusion: As shown above, the Ab-DeliverIN™ reagent does not cause mislocalization of antibodies upon delivery. The NLS-bearing Ab accumulates as expected in the nucleus.

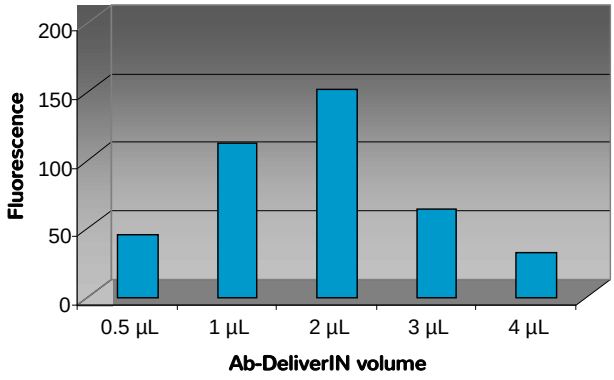


Several Quantitative Data

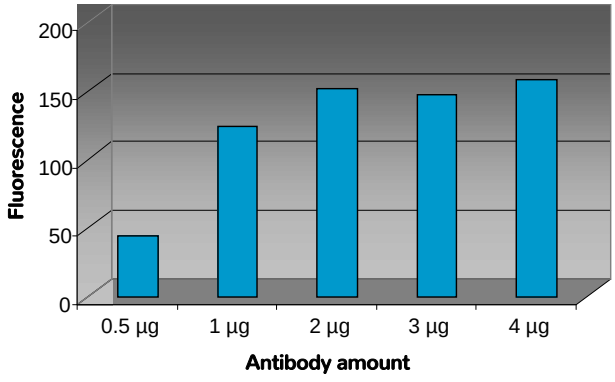
1- Dose-Response studies of antibody Delivery



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

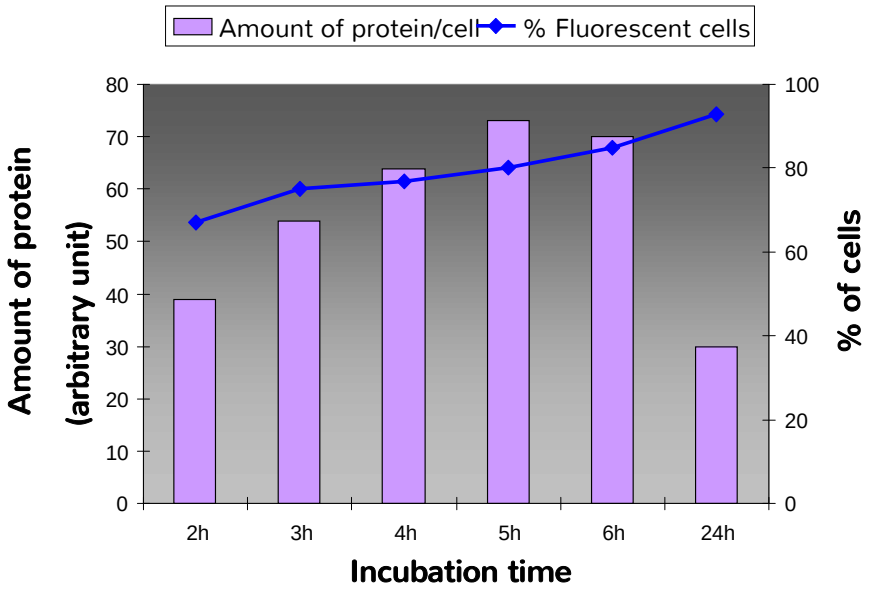


One µg of FITC-labeled Ab was delivered in NIH3T3 with the indicated amount of **Ab-DeliverIN™ Antibody Delivery Reagent** in 24-well plates. Cells were collected after 4h incubation time and the fluorescence level was monitored by spectrofluorimetry.



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

2- Kinetic of Ab delivery in NIH3T3 cells



One µg of FITC-labeled Ab was delivered in NIH3T3 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 was 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 Treatment with trypsine	No Yes	No No	Yes Yes	Yes No
% of IgG-FITC recovered in NIH3T3	**18**	**44**	**21**	**32**

Two µg of FITC-labeled Ab were delivered in NIH3T3 with 3 µL 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 incubation time 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. Results show that 18 % and 21 % of the input material was internalized in the absence or presence of FBS, respectively.

COS-7	**21**
HeLa	**8**
Jurkat	**0**
NIH3T3	**21**
Vero	**14**

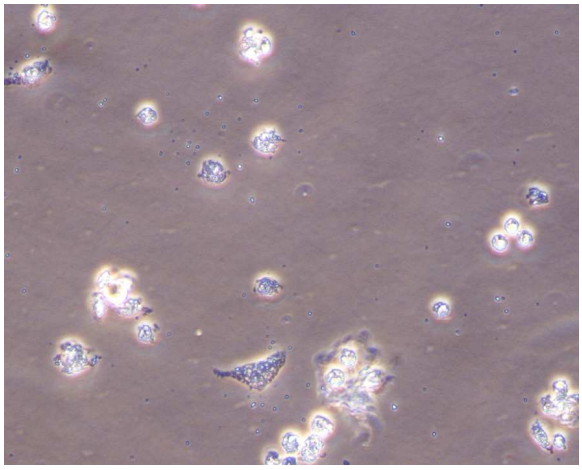
Percentage of cytosolic Ab-FITC internalized with different cell lines.

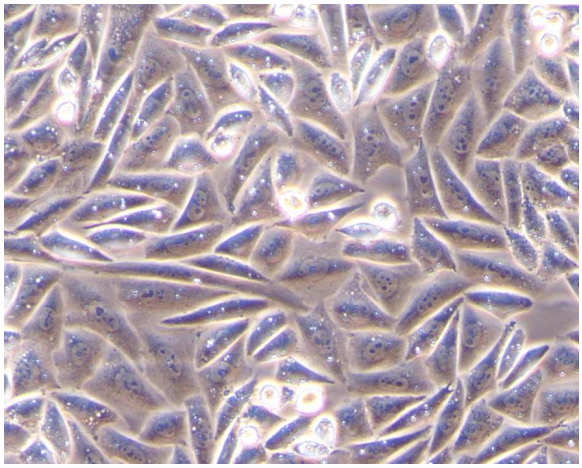
4- Delivery efficiency of Ab in various cell lines

Cells	% of positive cells
3T6	> 50 %
A549	50-80 %
B16-F10	> 50 %
BEAS-2B	80 %
BHK21	80-90 %
CHO-K1	50-80 %
COS-7, COS-1	50-70 %
HEK293	80-100 %
HeLa	50-60 %
L929	80-90 %
NIH3T3	50-75 %
Raw 264.7	90 %
U87	> 50 %
U937	80-90 %
Vero	> 50 %
K562	10-50 %
HaCaT	10-50 %
MDCK	10-50 %
Jurkat	< 5 %

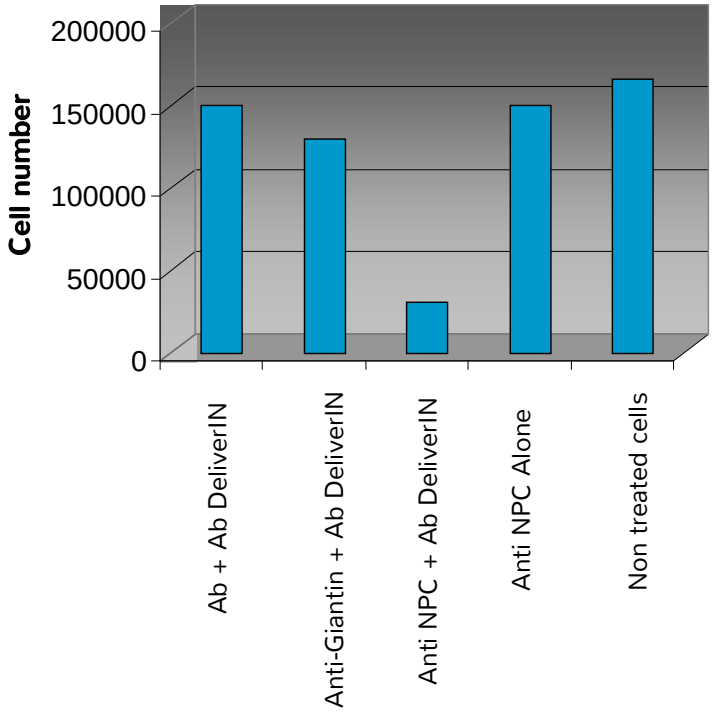
Primary Cells	% of positive cells
Neurons and glial cells	> 50 %

Anti-NPC Antibody Delivery and Biological Activity



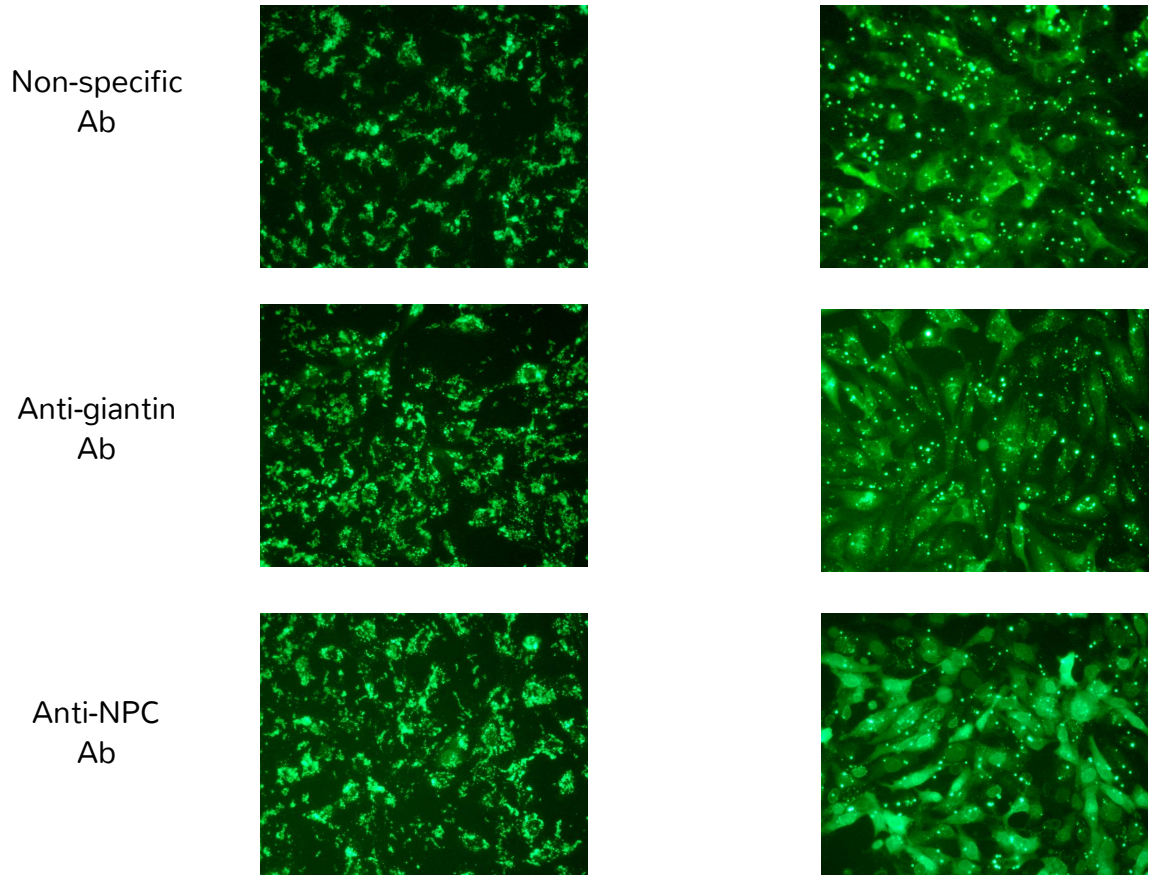


CHO-K1 cells were incubated with 0.5 µg of anti-NPC antibodies and 2 µL of **Ab-DeliverIN™** in 24 well plates (left picture) during 72 hours. As a control cells were incubated with **Ab-DeliverIN™** alone (not shown) or with **Ab-DeliverIN™** plus a non-specific antibody (right picture). Quantitative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



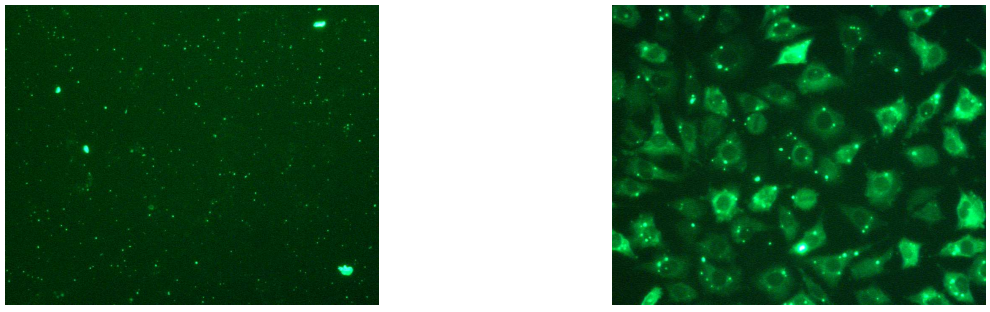
Conclusion: The delivery of the anti-NPC antibody with Ab-DeliverIN™ induces cell death. Although the mechanism of this cell death is unknown, cell division is required to obtain such results. An explanation could be that the anti-NPC antibody interferes with the nuclear envelope reconstitution after cell division. 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 a competitor



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 A** as described in its procedure manual or with our **Ab-DeliverIN™ reagent**.

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 its procedure manual or with our **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 **Ab-DeliverIN™.**