

Pro-DeliverIN™ - Protein Delivery Reagent Results

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

Main **Pro-DeliverIN™** features:

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

Protein Delivery

Delivery systems allowing exogenous proteins to be transported inside living cells represent a major interest. It opens novel strategies to assess functions of proteins or to elucidate new molecular mechanisms. Some approaches based on the use of PTD (Peptide Transduction Domain) 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 required. **Pro-DeliverIN™** is a formulation of lipids able to capture proteins through electrostatic and hydrophobic interactions and deliver them inside cells. Consequently, **Pro-DeliverIN™** delivers proteins in living cells without biochemical modification and these proteins remain intact in term of structure and function. The complexes formed are internalized by cells and are efficiently released into the cytoplasm. The **Pro-DeliverIN™** reagent / protein non-covalent complexes are fully biodegradable and non cytotoxic. Examples of potential applications are: 1) Intracellular localization studies in living cells, 2) Protein function, 3) Protein-protein interaction studies, 4) FRET studies...

Proteins Delivered

Several proteins were efficiently delivered in living cells with the **Pro-DeliverIN™** reagent. They were labeled or not with various *fluorophores* such as FITC, TRITC, Cy3. These proteins include:

- R-Phycoerythrin & B-Phycoerythrin
- Bovine Serum Albumin
- β -galactosidase
- Human caspase-3, caspase 8 and caspase 9
- Immunoglobulins
- MBP-fusion protein

It is highly critical that the protein of interest to be delivered is very pure. It is clear that any impurities, contaminants or additives present with your protein of interest might affect the delivery efficiency. Consequently, we suggest using a recombinant protein as pure as possible.

Cell Types Successfully Tested

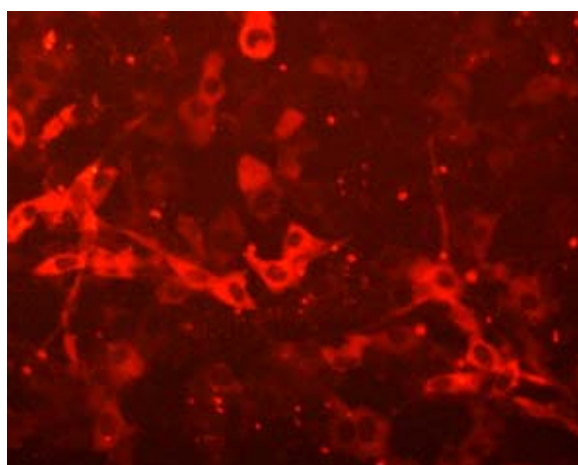
Pro-DeliverIN™ - Protein Delivery Reagent is applicable on numerous cell types. This reagent has been successfully tested on a variety of immortalized cell lines as well as some primary cells (see Table 1 page 2). An updated list of cells effectively tested is available on OZ Biosciences website: www.ozbiosciences.com.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

Table 1: Example of cells successfully assayed with **Pro-DeliverIN™** reagent.

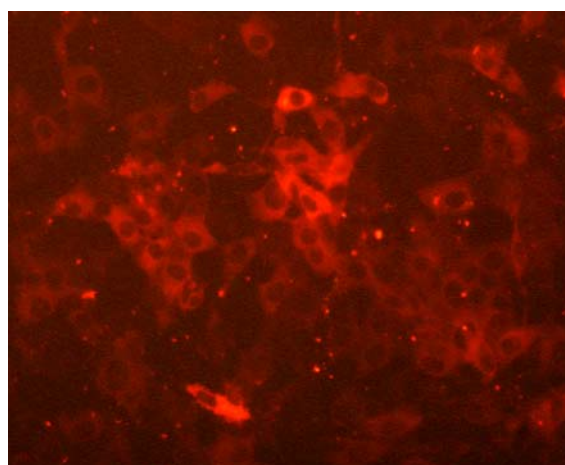
Cell Line	Cell Type	Source
3T6	Embryonic fibroblasts	Mouse
A549	Non-small cell lung carcinoma	Human
B16-F10	Melanoma	Mouse
BEAS-2B	Bronchial epithelial cells	Human
BHK21	Fibroblasts (Kidney)	Hamster
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblasts (Kidney)	Green Monkey
HaCaT	Keratinocytes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

B and R-Phycoerythrin Delivery

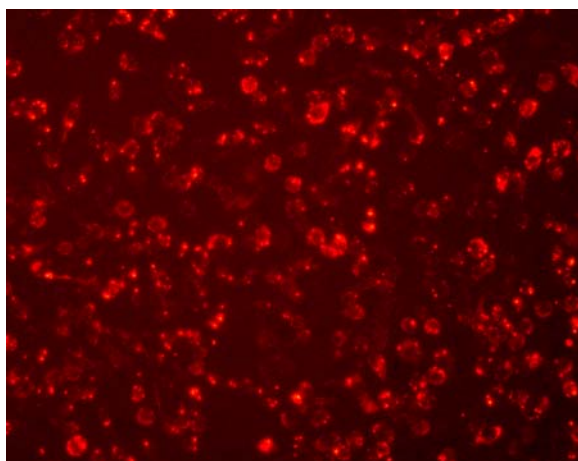
NIH3T3



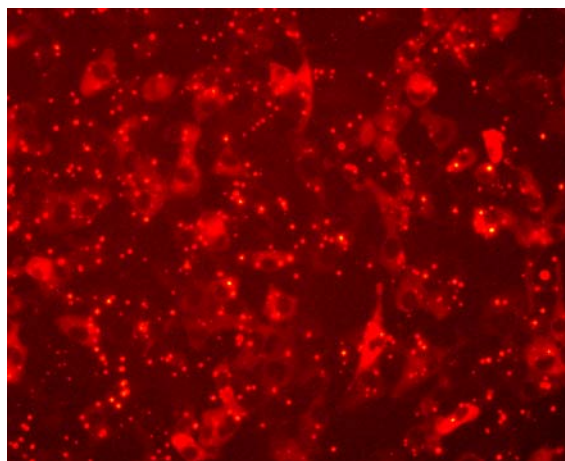
A549



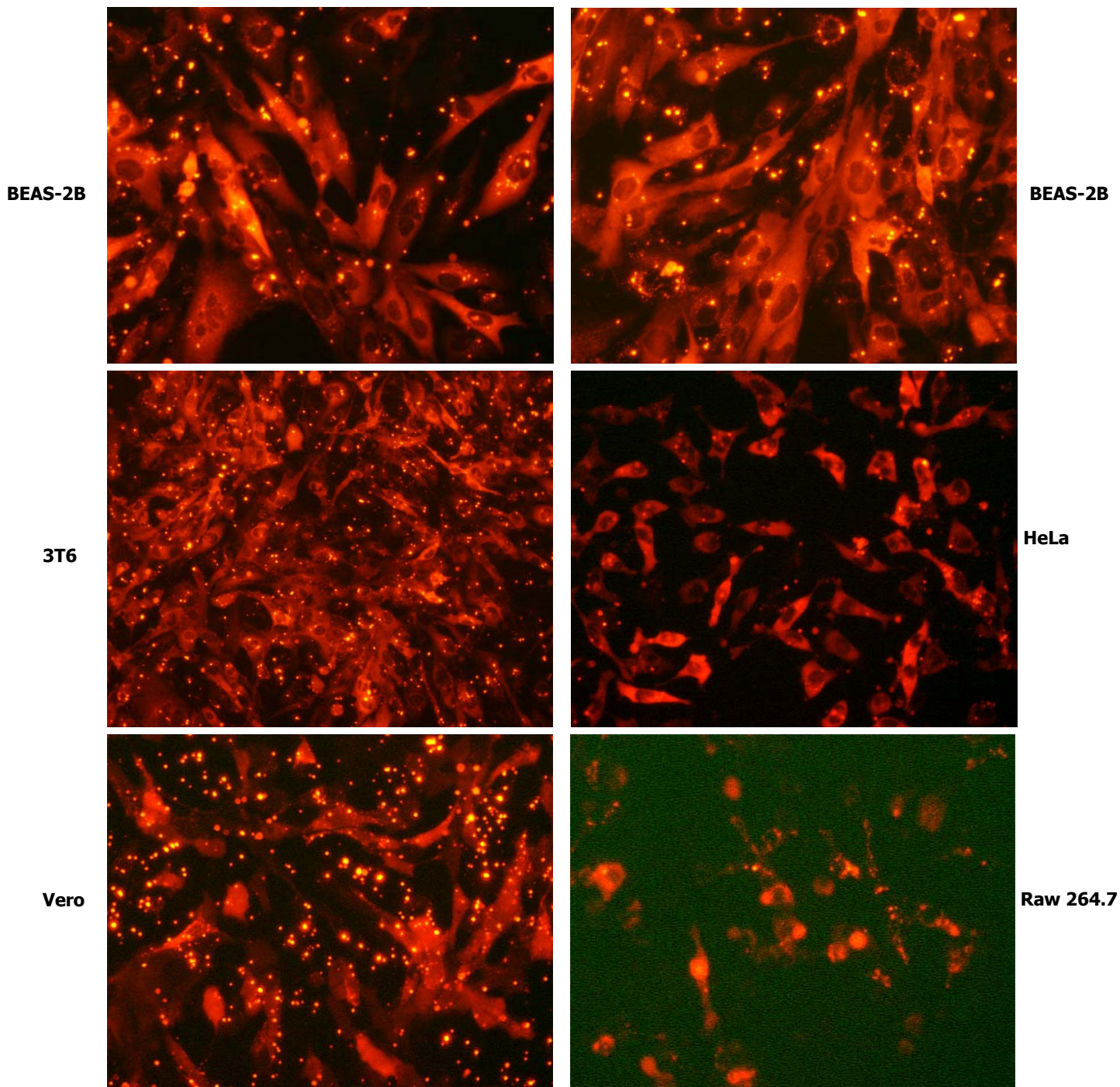
RAW 264.7



BHK21



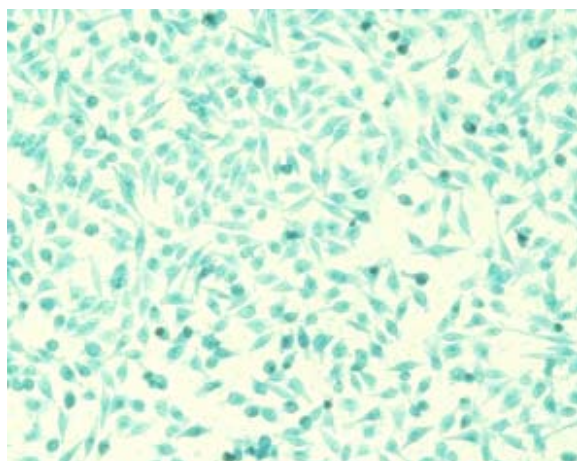
B-Phycoerythrin (1 μg , Sigma-Aldrich) was delivered in the indicated cell lines with 2 μL of **Pro-DeliverIN**[™] reagent. Phycoerythrin / **Pro-DeliverIN**[™] complexes were incubated 24 hours in 24-well plates, and then live cells were observed by fluorescence microscopy.



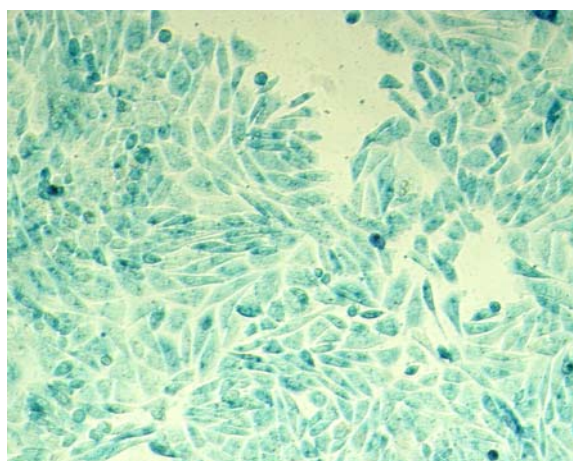
R-Phycoerythrin (1 μg , Molecular probes) was delivered in the indicated cell lines with 2 μL of **Pro-DeliverIN**[™] reagent. Phycoerythrin / **Pro-DeliverIN**[™] complexes were incubated 24 hours in 24-well plates. Live cells were observed by fluorescence microscopy.

β-Galactosidase Delivery

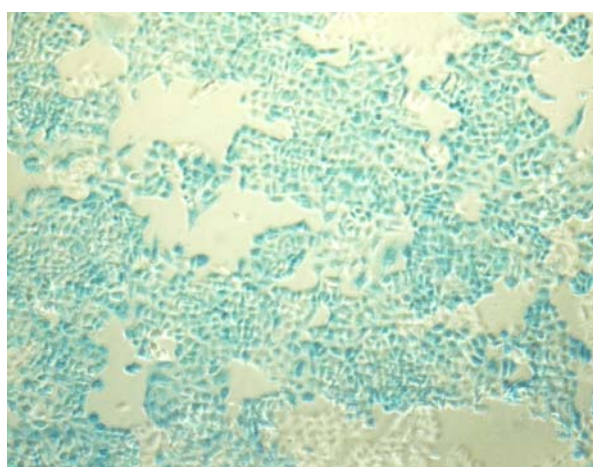
HeLa



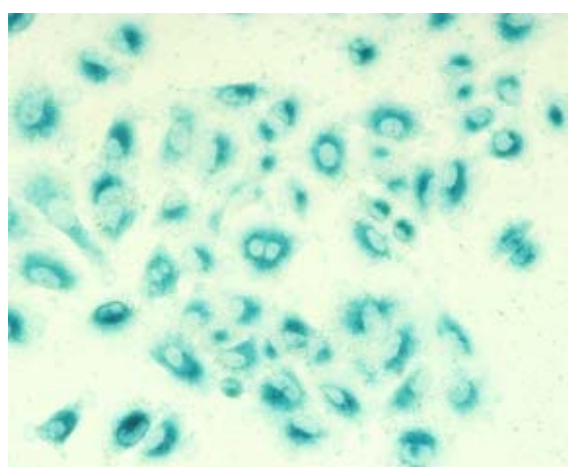
CHO-K1



A549



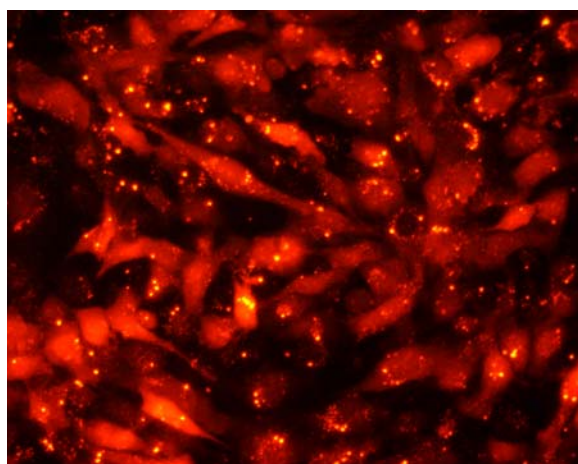
A549



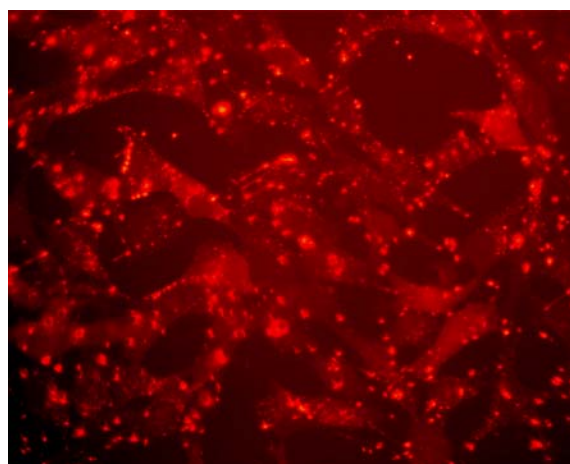
1 μ g of β -Galactosidase was delivered in various cells with 2 μ L of the **Pro-DeliverIN™** reagent. β -Galactosidase / **Pro-DeliverIN™** complexes were incubated 24 hours in 24-well plates and then cells were fixed and stained with X-Gal staining kit (OZ Biosciences catalog # GX10003).

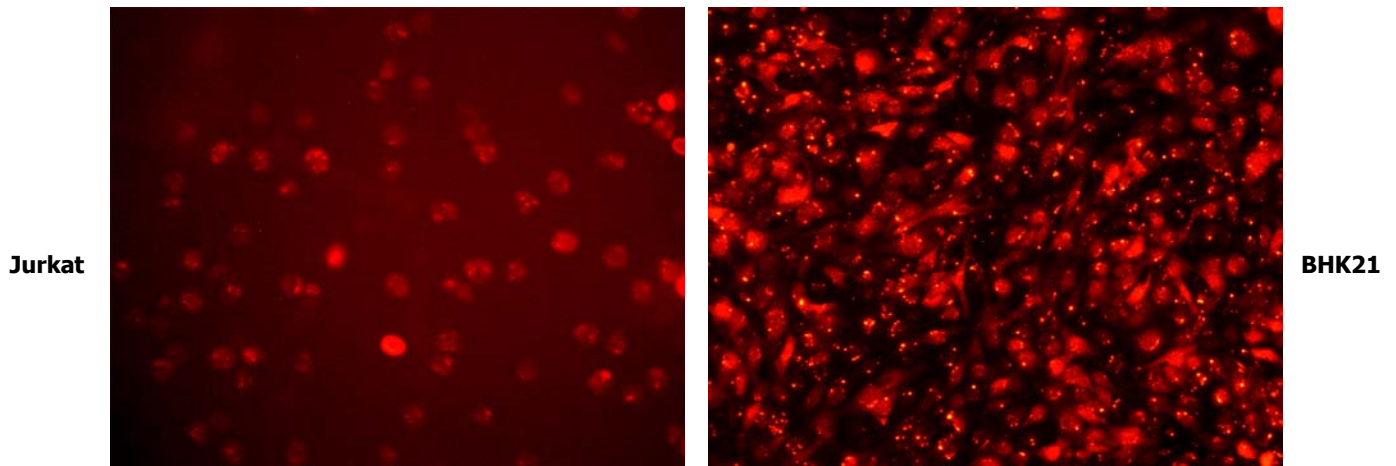
BSA-TRITC Delivery

BEAS-2B



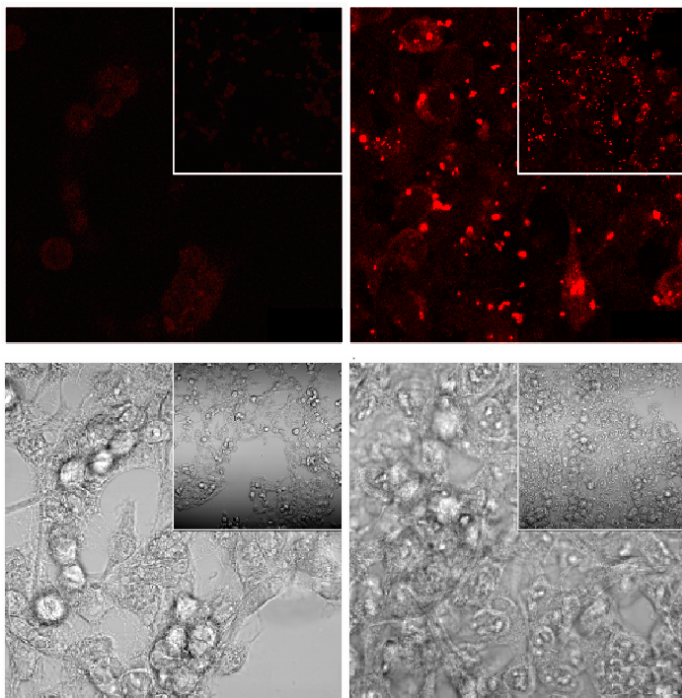
NIH3T3





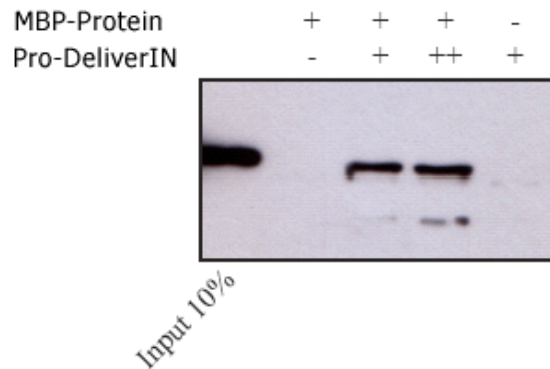
2 μg of Tetramethylrhodamine labeled BSA (BSA-TRITC) were delivered in various cells with 3 μL of the **Pro-DeliverIN™** reagent. BSA-TRITC / **Pro-DeliverIN™** complexes were incubated 24 hours on cells in 24-well plates and then living cells were observed with a fluorescent microscope.

MBP- protein Delivery

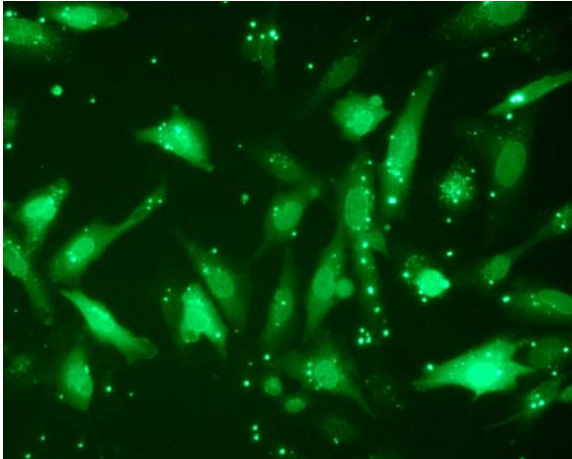


MBP-Protein alone

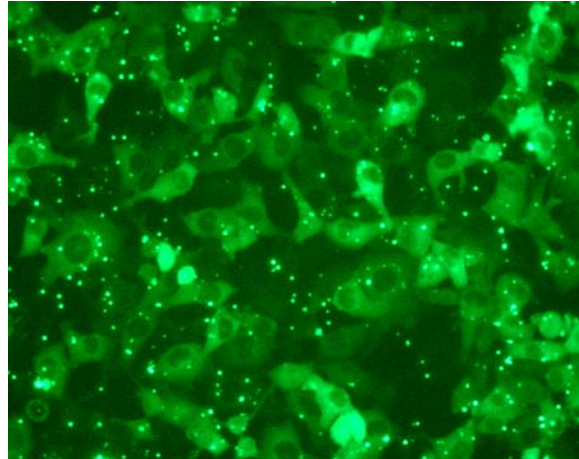
MBP-Protein delivery with the **Pro-DeliverIN™** reagent



The MBP-fusion protein (10 μg) was delivered with 25 μL of the **Pro-DeliverIN™** reagent in HEK 293 cells. After 8 h of incubation, cells were fixed and immunostained with anti-MBP antibody. Then cells were observed by fluorescence microscopy. After 10 h of incubation cells were lysed for protein detection by western-blot. These results were kindly provided by Dr J. Weiske (Charité CBF, Berlin, Germany).

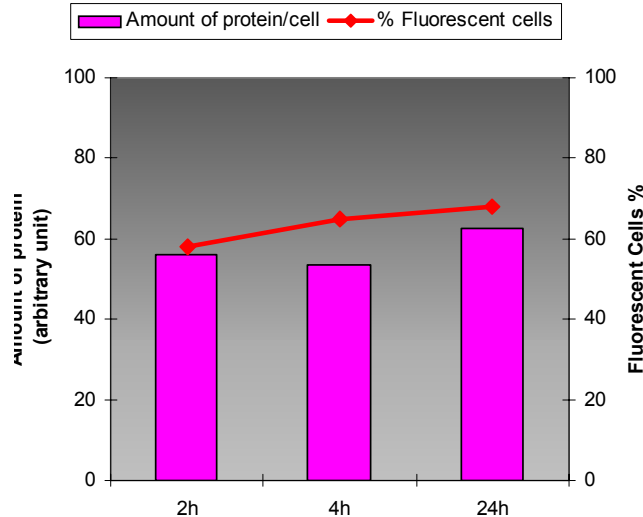


0.5µg of a fluorescently labeled antibody (AlexaFluor®488) directed against the Nuclear Pore Complex proteins was mixed with 2µL of the **Pro-DeliverIN™** reagent and incubated 24 hours on BEAS-2B cells in a 24-well plate. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.



0.5µg of a fluorescently labeled antibody (AlexaFluor®488) was mixed with 2µL of the **Pro-DeliverIN™** reagent and incubated 24 hours on BHK-21 cells in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.

Kinetic of R-Phycoerythrin delivery in NIH3T3 cells

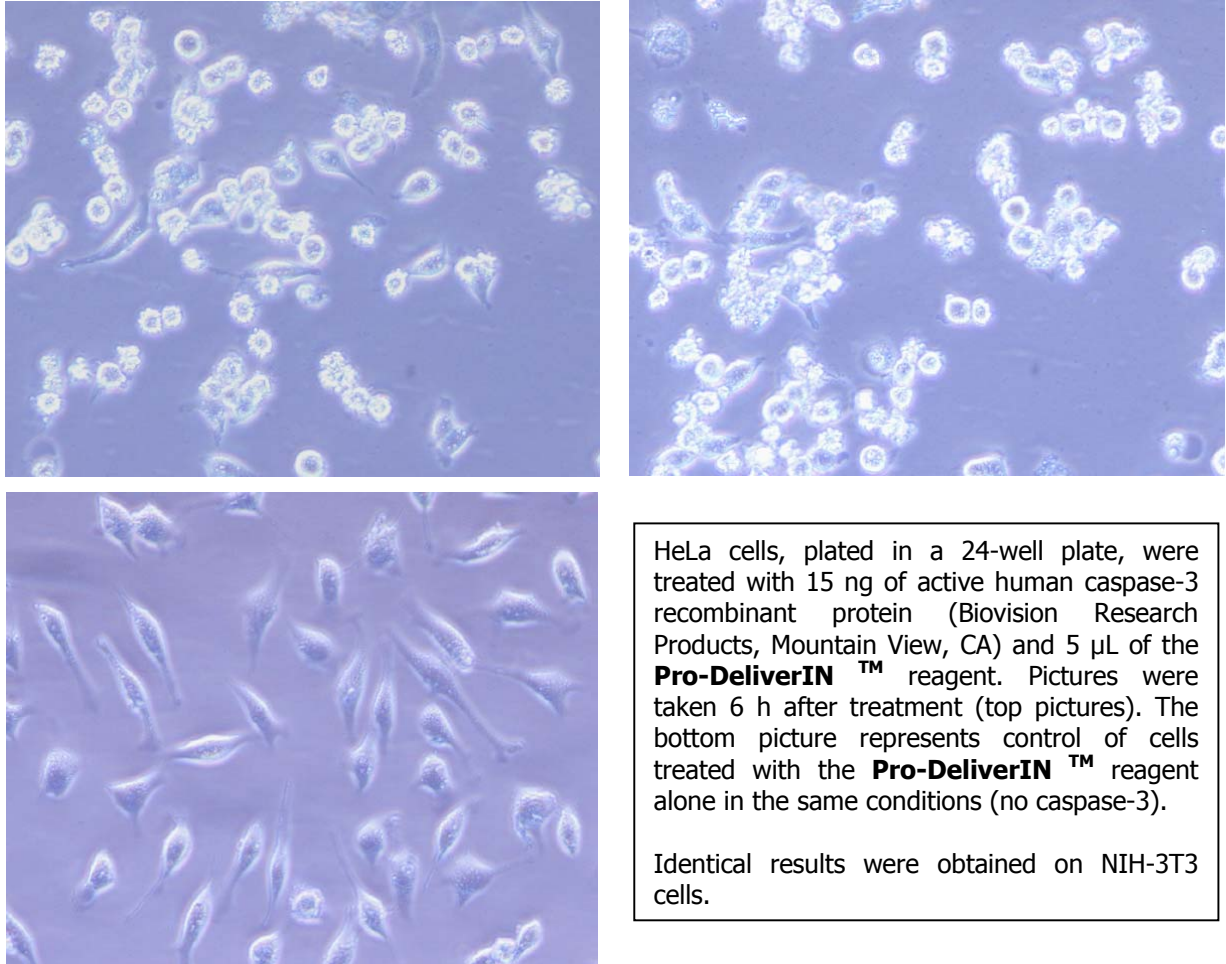


One µg of R-Phycoerythrin was delivered in NIH3T3 cells with 2 µL of the **Pro-DeliverIN™** reagent in 24-well plates. Cells were collected and fixed with 2% PFA at the indicated time point. The number of fluorescent cells and the mean fluorescence was determined by cytofluorimetry. The mean fluorescence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

Conclusion: Various Proteins were efficiently delivered in a large number of living cells. The efficiency is cell type and protein dependant. It is important to note that acidic proteins are delivered much more efficiently than basic proteins.

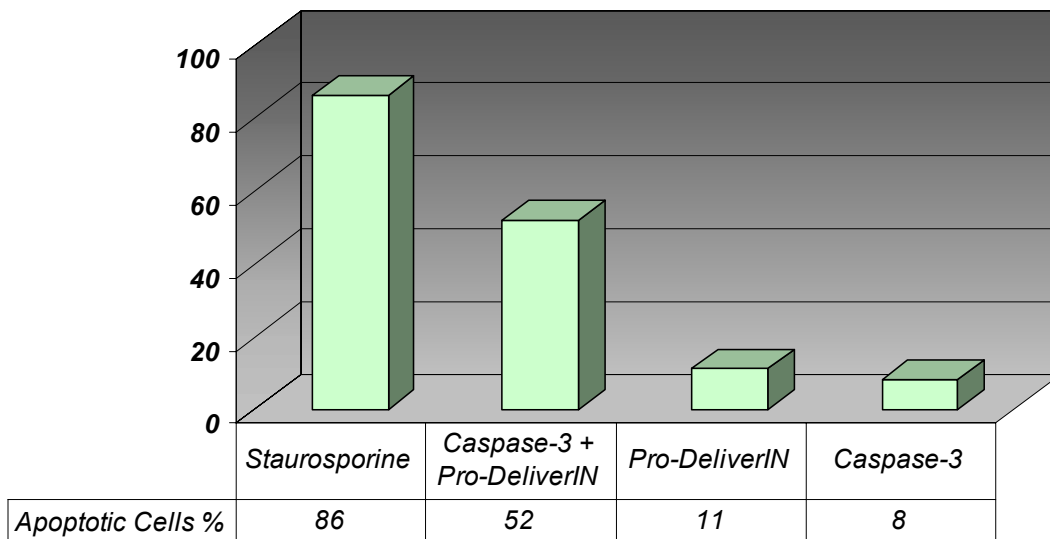
Active Caspase-3 Delivery and Induction of Apoptosis

As shown previously with the β -galactosidase, the proteins are still active upon delivery. However, in order to study if the protein delivered can exert its functions and influence cell processes, we set up an apoptosis assay using an active human caspase-3 recombinant protein.

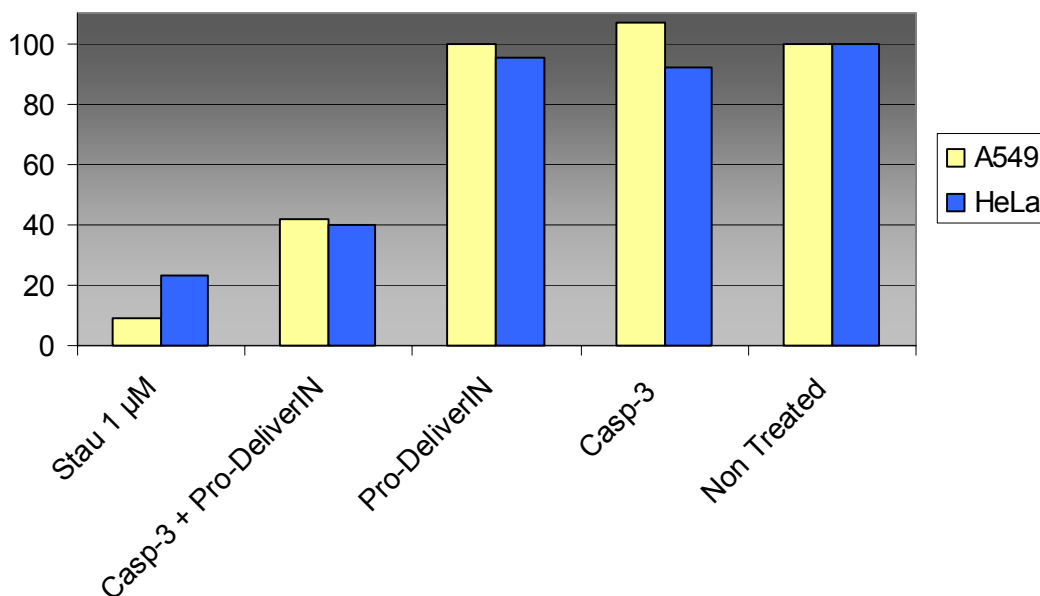


HeLa cells, plated in a 24-well plate, were treated with 15 ng of active human caspase-3 recombinant protein (Biovision Research Products, Mountain View, CA) and 5 μ L of the **Pro-DeliverIN**™ reagent. Pictures were taken 6 h after treatment (top pictures). The bottom picture represents control of cells treated with the **Pro-DeliverIN**™ reagent alone in the same conditions (no caspase-3).

Identical results were obtained on NIH-3T3 cells.



HeLa cells were treated with 15 ng of active human caspase-3 and 5 μ L of the **Pro-DeliverIN™** reagent in 24 well plates. As controls, cells were treated either with 15 ng of caspase 3 alone or with 5 μ L of **Pro-DeliverIN™** alone. As a positive control, staurosporine (100 nM) was used to induce apoptosis. After 7 h of incubation, cells were stained with both Annexin-FITC and propidium iodide. Apoptotic and dead cells were monitored by cytofluorimetry.



HeLa and A549 cells were treated with 15 ng of active human recombinant caspase-3 and 5 μ L of **Pro-DeliverIN™** reagent in 24 well plates. As controls, cells were treated either with 15 ng of caspase-3 alone or 5 μ L of the **Pro-DeliverIN™** reagent alone. As a positive control, staurosporine (1 μ M) was used to induce apoptosis. After 24h incubation, cells were counted in each well and results were presented as relative amount of cells compare to non-treated cells.

Conclusion: The delivery of the human caspase-3 recombinant protein with the Pro-DeliverIN™ reagent allows the induction of apoptosis.