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

Main features are:

1. Efficient protein delivery in a wide variety of cells.
2. Various proteins were delivered into 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.

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, the proteins delivered inside cells with Pro-DeliverIN™ retain their structure and function. The complexes formed are internalized by cells and are efficiently released into the cytoplasm without any cytotoxicity.

Cell Types Successfully Tested

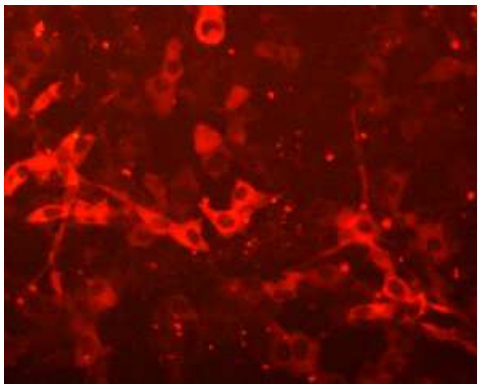
Pro-DeliverIN™ Protein Delivery Reagent is applicable on numerous cell types. This reagent has been tested on several cell lines and OZ Biosciences is maintaining an updated list of cells successfully tested that is available on the website: www.ozbiosciences.com. If a particular cell type is not listed,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

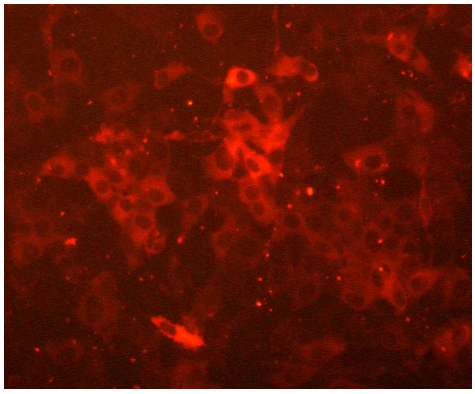
Proteins Delivered

Several proteins were efficiently delivered in living cells with the **Pro-DeliverIN™ Protein Delivery Reagent.** These proteins include R-Phycoerythrin, BSA-TRITC, β-galactosidase, the human caspase-3 protein and immunoglobulins.

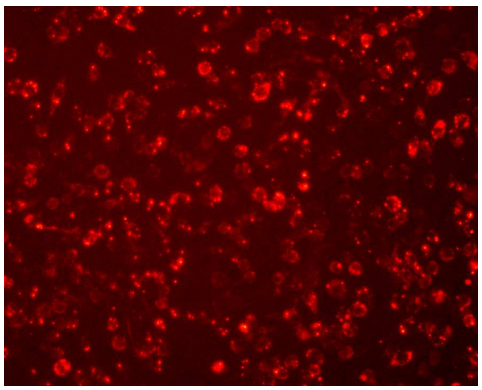
B and R-Phycoerythrin Delivery



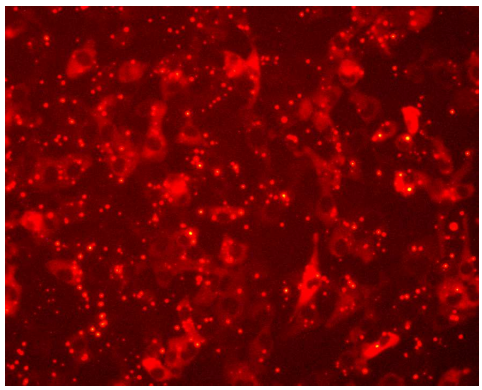
NIH3T3



A549

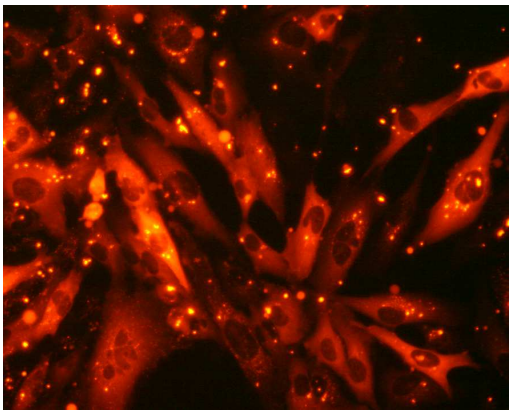


RAW 264.7

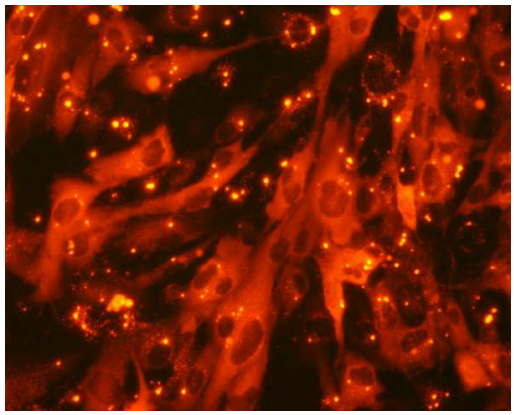


BHK21

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



BEAS-2B



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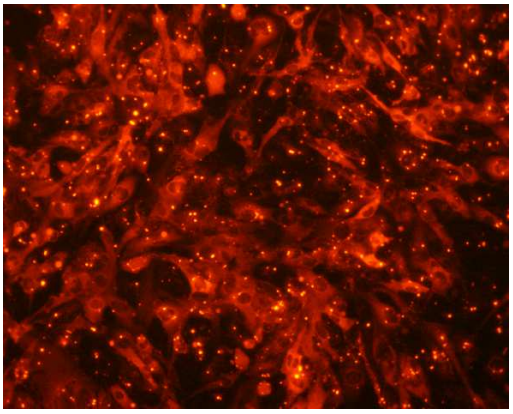
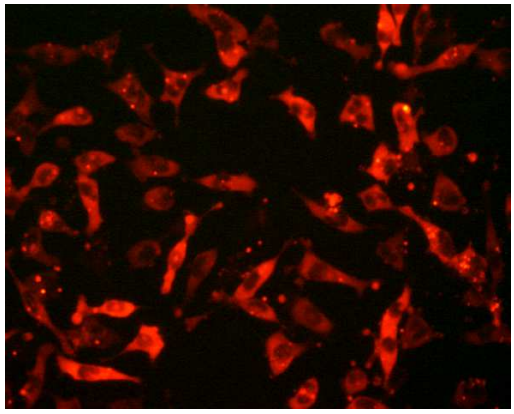
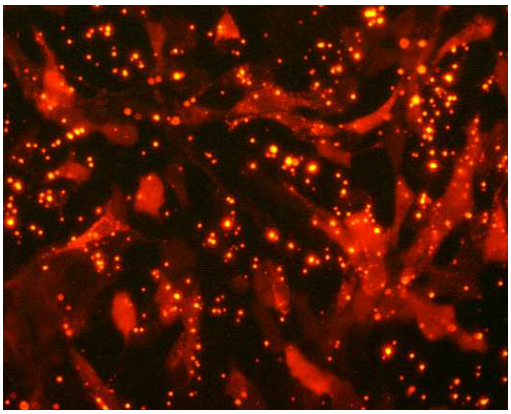


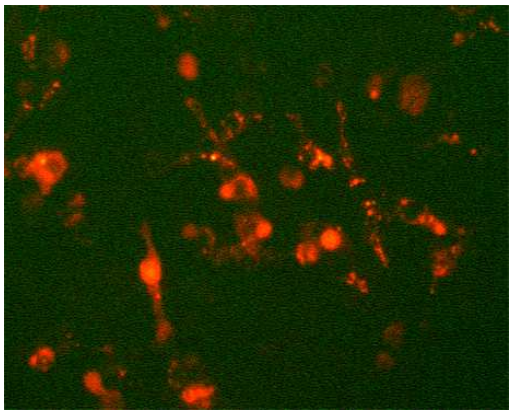
3T6



HeLa



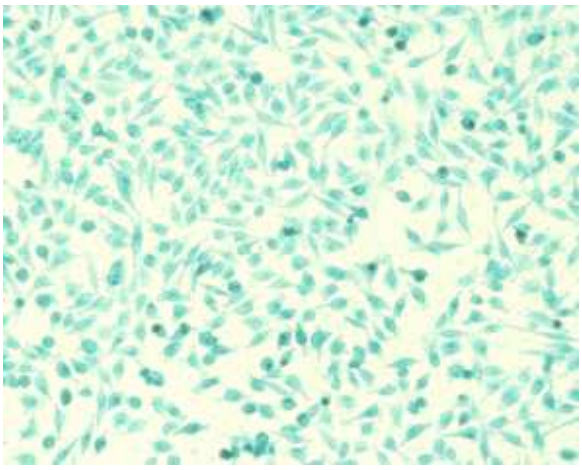
Vero



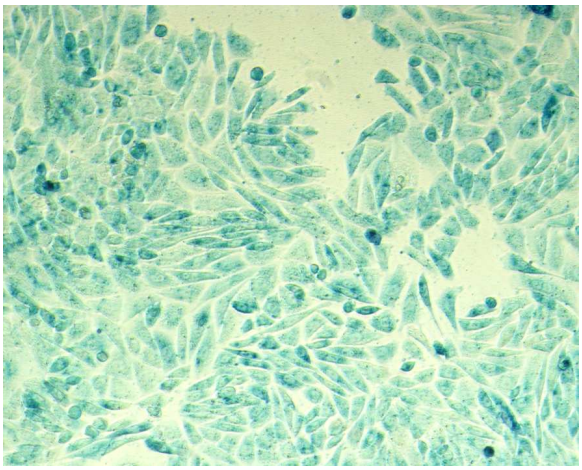
Raw 264.7

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

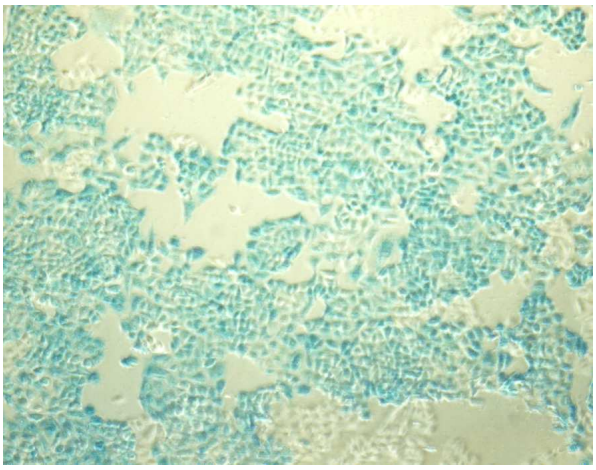
β-Galactosidase Delivery



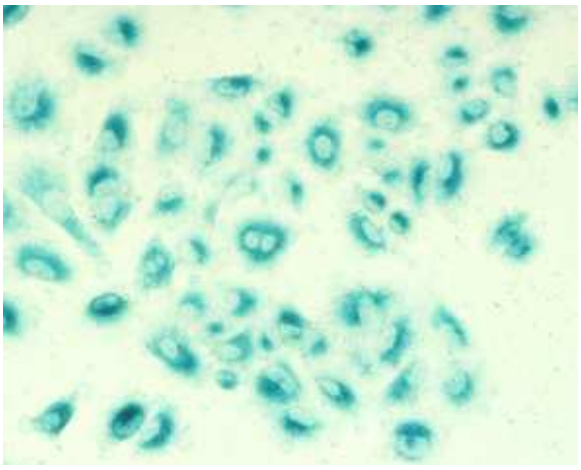
HeLa



CHO



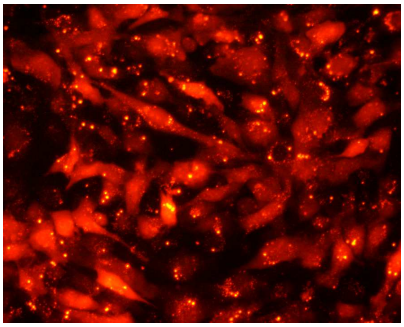
A549



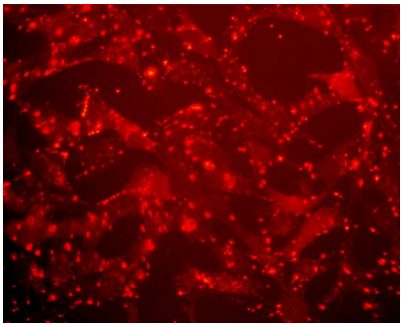
A549

β-Galactosidase (1 µg) was delivered in the indicated cells. β-Galactosidase-**Pro-DeliverIN™** complexes were incubated 24 hours in 24-well plates. Cells were fixed and stained with X-Gal (OZ Biosciences).

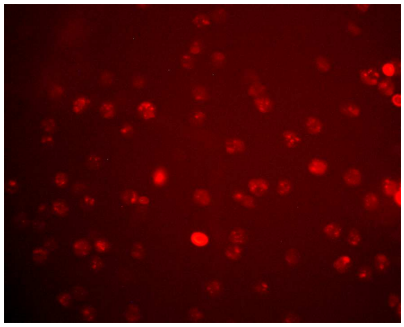
BSA-TRITC Delivery



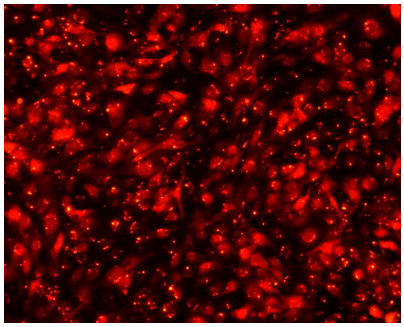
BEAS-2B



NIH3T3



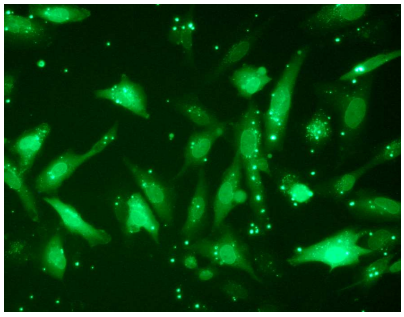
Jurkat



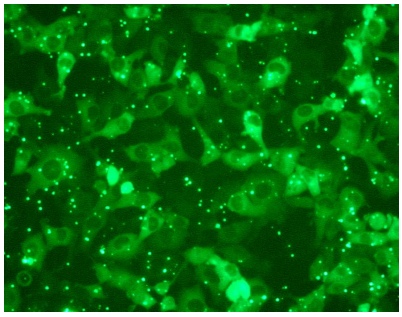
BHK21

BSA-TRITC (2 µg) was delivered in the indicated cells with 3 µL of **Pro-DeliverIN™**. Tetramethyl rhodamine labeled BSA-**Pro-DeliverIN™** complexes were incubated 24 hours in 24-well plates. Cells were fixed and stained with X-Gal (OZ Biosciences).

Immunoglobulins Delivery



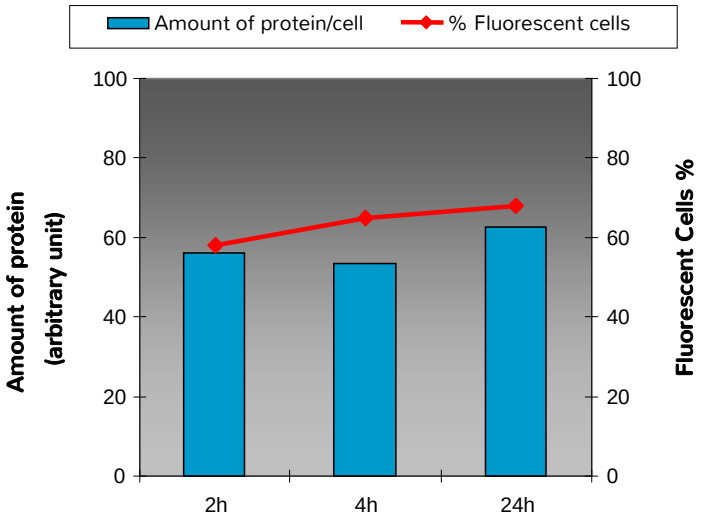
Ab-AlexaFluor®488 directed against Nuclear Pore Complex proteins (0.5 µg) was mixed with **Pro-DeliverIN™** (2 µL) and incubated 24 hours with BEAS-2B in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.



Ab-AlexaFluor®488 labeled (0.5 µg) was mixed with **Pro-DeliverIN™** (2 µL) and incubated 24 hours with BHK21 in 24-well plates. Cells were then fixed with 2% PFA and observed by fluorescence microscopy.

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

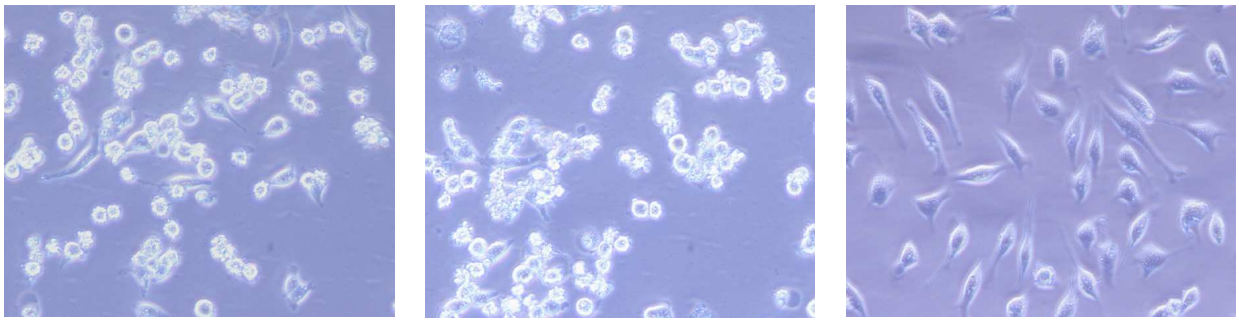
Kinetic of R-Phycoerythrin delivery in NIH3T3 cells



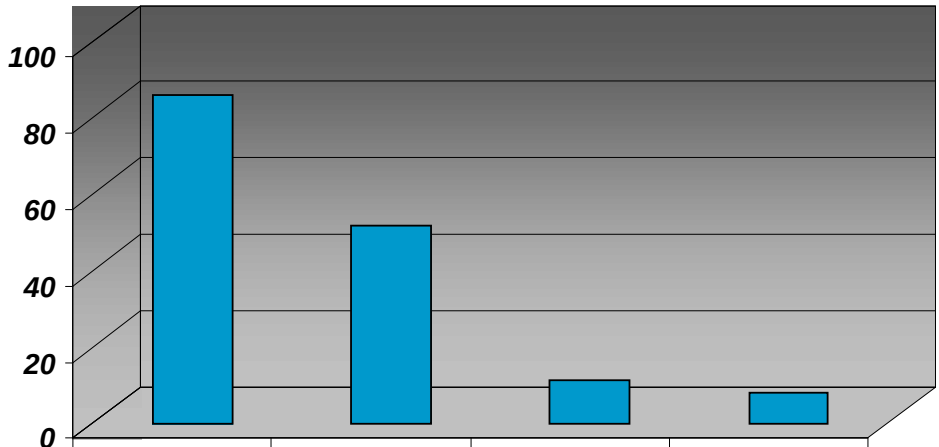
One µg of R-Phycoerythrin was delivered in NIH3T3 with 2 µL of **Pro-DeliverIN™ Protein Delivery Reagent** in 24-well plates. Cells were collected and fixed with 2% PFA at the indicated time point. The number of fluorescent cells and the mean fluorescence was determined by cytofluorimetry. The mean fluorescence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

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 function and influence cell processes, we set up an apoptosis assay using an active human caspase-3 recombinant protein.

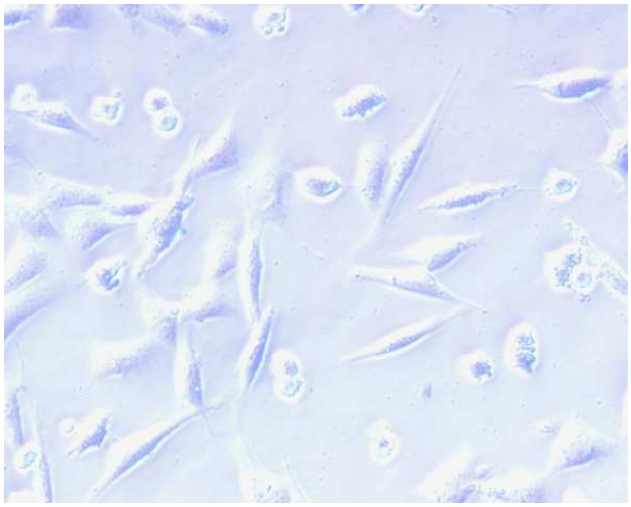


HeLa cells were treated with 15 ng of active human caspase-3 recombinant protein (Biovision Research Products, Mountain View, CA) and 5 µL of **Pro-DeliverIN™ Protein Delivery Reagent** in 24-well plates. Pictures were taken after 6h treatment (top pictures). The bottom picture represents cells treated with the Pro-DeliverIN™ reagent in the same conditions but the caspase-3 was omitted.



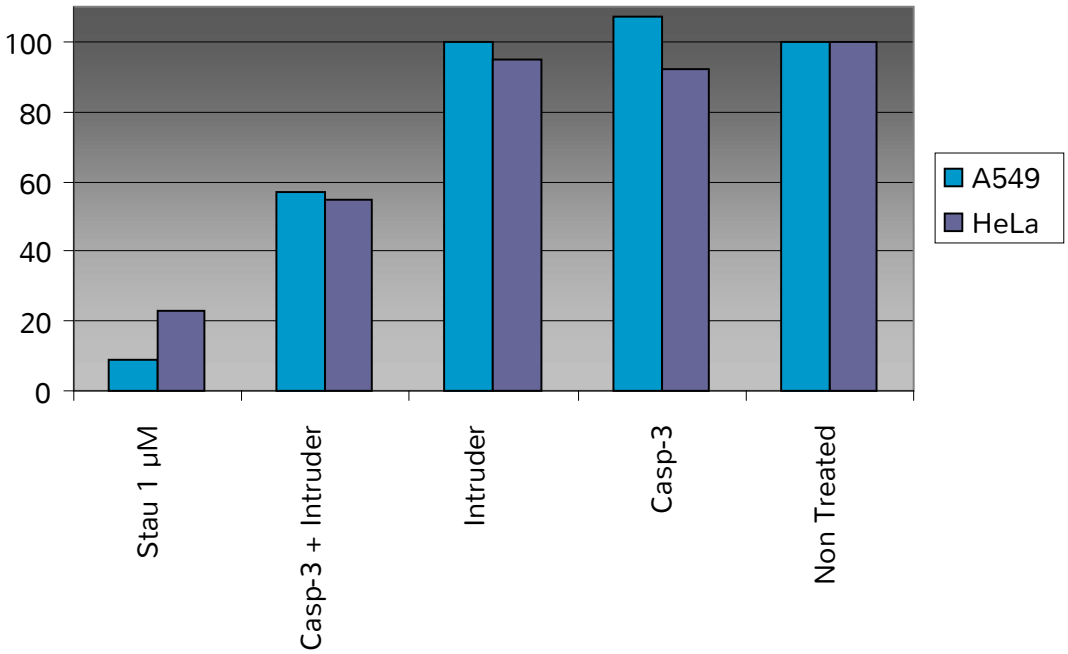
	Staurosporine	Caspase-3 + Intruder	Intruder	Caspase-3
Apoptotic Cells %	86	52	11	8

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



NIH3T3 cells were treated with 15 ng of active human caspase-3 recombinant protein (Biovision Research Products, Mountain View, CA) and 5 µL of **Pro-DeliverIN™** in 24-well plates. Pictures were taken after 6h treatment.

NIH3T3 cells were treated with 5 µL of **Pro-DeliverIN™** and no caspase-3 in 24-well plates. Pictures were taken after 6h treatment.



Hela and A549 cells were treated with 15 ng of active human caspase-3 and 5 µL of **Pro-DeliverIN™ Protein Delivery Reagent** in 24 well plates. As controls cells were treated either with 15 ng of caspase 3 or 5 µL of **Pro-DeliverIN™** alone. As a positive control, staurosporine (1 µM) 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 with Pro-DeliverIN™ of the human caspase-3 recombinant protein allows inducing cell apoptosis.