

# **PULSin™ *in vitro* Protein, Antibody and Peptide Delivery Protocol**

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## Company Information

### Technical Assistance and Scientific Advice

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## Product Information

PULSin™ is a novel reagent dedicated to the transfer of peptides, antibodies and proteins into cells. It contains a cationic amphiphile molecule whose formulation is proprietary. PULSin™ delivers anionic proteins and antibodies to a large variety of eukaryotic cell lines including primary cells (Table 1). PULSin™ is most efficient when able to interact with the protein by electrostatic and/or lipophilic interactions. Thus, anionic proteins (i.e. proteins with an isoelectric point < 7) and antibodies are particularly well-suited for delivery with PULSin™. Yet delivery is not restricted to anions, as most proteins have a lipophilic core.

### Ordering information

Cat #	PULSin™ Reagent	Number of delivery experiments
501-01	0.1 ml	6 delivery experiments in 6 well-plate
501-04	0.4 ml	25 delivery experiments in 6 well-plate
501-16	4 x 0.4 ml	100 delivery experiments in 6 well-plate

### Content

- PULSin™ delivery reagent,
- R-phycoerythrin (R-PE) (20 µg) to be used as a positive control at 0.1 µg/µl. The excitation of R-PE by 488 nm laser light induces a light emission maximum of 575 nm,
- HEPES Buffer (20 mM), 20 ml (cat # 501-01 and 501-04) or 4 x 20 ml (cat # 501-16) for protein dilution.

0.4 ml of PULSin™ reagent is sufficient to perform ca. 100 experiments in 24-well plates or ca. 25 experiments in 6-well plates.

### Reagent use and Limitations

For research use only. Not for use in humans.

### Quality control

Functional analysis: Every batch of PULSin™ is tested by delivering R-phycoerythrin into HeLa cells. The batch is validated if 1 µg of positive control protein gives at least 70% of fluorescent cells when measured by FACS analysis.

### Formulation and Storage

PULSin™ is provided as an aqueous solution in sterile and apyrogenic water. PULSin™, R-phycoerythrin and HEPES buffer is shipped at 4°C and should be stored at 4°C upon arrival. PULSin™, R-phycoerythrin and HEPES buffer, as guaranteed by the Certificate of Analysis, will be stable for at least one year when stored appropriately.

## 1. Delivery protocol for adherent cells

### 1.1 Cell seeding

For optimal protein delivery with PULSin™, the cells should be 70-80% confluent on the day of the experiment. Typically, for protein delivery in 24-well plates, 70 000 to 100 000 cells are seeded per well and incubated for 16 to 24 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Refer to Table 1 for protein delivery in other culture formats. Since the efficiency of protein delivery depends partly on cell confluency, the culture density should be optimized for each new cell line.

**Table 1. Recommended number of cells to seed 1 day before protein delivery with PULSin™**

Culture Vessel	Surface area per well (cm <sup>2</sup> )	Number of adherent cells to seed	Volume of medium per well (ml)
96-well	0.3	15 000 - 25 000	0.2
48-well	1	30 000 - 50 000	0.5
24-well	1.9	70 000 - 100 000	1
12-well	3.8	140 000 - 200 000	2
6-well / 3.5 cm	9.4	300 000 - 400 000	3
6 cm / flask 25 cm <sup>2</sup>	28	600 000 - 800 000	5
10 cm / flask 75 cm <sup>2</sup>	79	1.10 <sup>6</sup> - 2.10 <sup>6</sup>	10

### 1.2 Preparation of protein/PULSin™ complexes and delivery procedure

This protocol (Figure 1) is given for the delivery of 1 µg of protein, antibody or peptide.

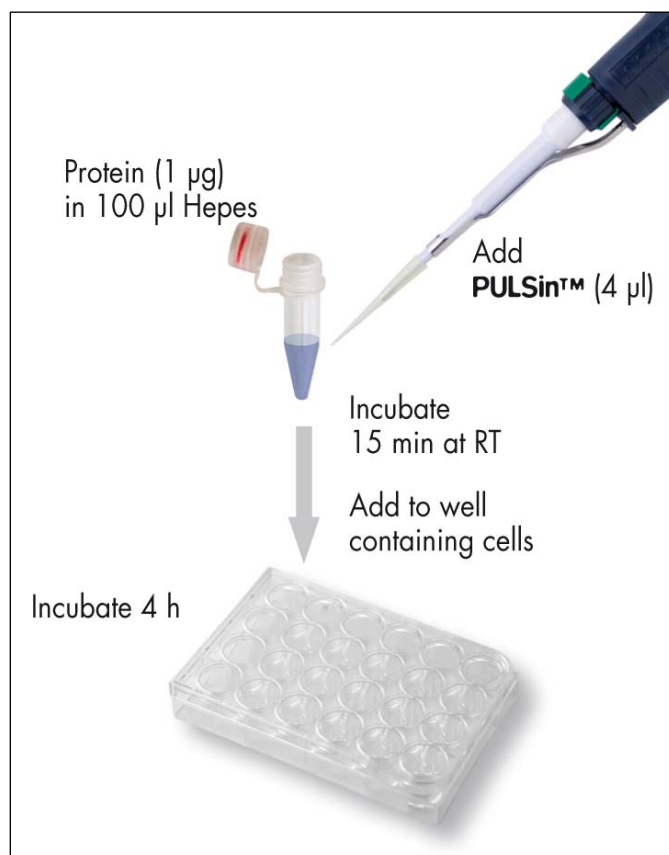
- For R-PE, use 4 µl of PULSin™ per µg of protein.
- For antibodies, start with 2.5 µl of PULSin™ per µg of antibody.

Guidelines for optimization are given in section 1.3. Table 2 gives experimental conditions for other cell culture plate formats.

## Per well in 24 well-plate

1. Dilute 1 µg of protein in 100 µl of 20 mM Hepes in a microcentrifuge tube. Vortex gently and spin down briefly.
2. Add 4 µl of PULSin™. Vortex immediately and spin down briefly.
3. Incubate for 15 minutes at room temperature. Wash cells once with 1X PBS or culture medium without serum. The washing step is critical to remove all traces of serum.
4. Add 900 µl of culture medium without serum.
5. Add 100 µl of PULSin™/protein mix per well and homogenize by gently swirling the plate.
6. Incubate at 37°C in a 5% CO<sub>2</sub> incubator.
7. After 4 hours, remove the medium containing the biomolecules/PULSin™ complexes and replace with fresh complete medium.
8. Analyze the cells immediately or after an incubation period. Delivery can be analyzed by assessing protein activity or visualizing intracellular fluorescence.

**N.B.:** for R-PE, the excitation by a laser at 488 nm induces a light emission maximum of 575 nm.



**Figure 1. PULSin delivery protocol for 24-well plate (per well)**

**Table 2. Experimental conditions of biomolecule delivery depending on cell culture vessel**

Culture Vessel	Amount of biomolecule (µg)	Volume of 20 mM Hepes buffer (µl)	Volume of PULSin™ (µl)
96-well	0.3	20	1.2
24-well	1	100	4
12-well	2	150	8
6-well / 3.5 cm	4	200	16
6 cm / flask 25 cm <sup>2</sup>	7	400	28
10 cm / flask 75 cm <sup>2</sup>	10	1000	50

### 1.3 Optimization guidelines

To obtain the best possible efficiency of delivery for the protein of interest with PULSin™, optimization is highly recommended. Moreover with the same protein, variations may be observed from one cell line to another. Thus we recommend testing a range of 0.5 µg to 4 µg of protein, antibody or peptide and 1 µl to 4 µl of PULSin™ (Table 3).

**Table 3. Optimization guidelines for PULSin™-mediated biomolecule delivery**

Amount of biomolecule (µg)	0.5			2			4		
Amount of PULSin™ (µl)	1	2	4	1	2	4	1	2	4

The amounts to be tested are the same for proteins, antibodies or peptides.

### 1.4 Delivery procedure for sensitive cells

Fragile cells may not withstand the absence of serum for 4 hours, thus we suggest an alternative protocol below. Proceed with Steps 1 to 5 as in 1.2, then add a short centrifugation step as indicated below.

1. Gently centrifuge the cells for 5 min at 190 g (if the cells can withstand it) directly after adding the PULSin™/protein complexes onto the cells.
2. Incubate for 30 min at 37°C in a 5% CO<sub>2</sub> incubator. Remove the medium containing the protein/PULSin™ complexes by aspiration and replace with fresh complete medium.
3. Process cells and analyze protein activity or localization immediately or after longer incubation time.

## 2. Delivery protocol for suspension cells

This protocol is given for protein delivery in 24-well plate using 500 000 cells grown in suspension in 1 ml of medium.

### 2.1 Cell seeding

For protein delivery in suspension (Jurkat, THP-1, K-562, etc.), cells are counted and collected by centrifugation (190g for 5 minutes) on the day of the experiment. Per 24-well plate, seed  $5 \times 10^5$  cells in 1 ml of Opti-MEM® I without serum but containing glutamine in a 10 ml sterile cell culture tube. The following protocol is given as a starting point, for optimization please refer to section 1.3.

### 2.2 Preparation of protein/PULSin™ complexes and delivery procedure

100  $\mu$ l of protein/PULSin™ complexes are required per 24-well plate and are prepared as follows:

1. Dilute 1 - 2  $\mu$ g of biomolecule into 100  $\mu$ l of 20 mM HEPES. Vortex gently and spin down briefly.
2. Add 2 - 4  $\mu$ l of PULSin™ into each tube. Vortex immediately and spin down briefly.
3. Incubate for 15 minutes at room temperature and add the protein/PULSin™ mix into the 1 ml of cells at the density of  $4 \times 10^5$ .
4. Incubate for 4 hours, at 37°C in a 5% CO<sub>2</sub> humidified atmosphere, preferentially under agitation.
5. Transfer to a new tube and pellet the cells by centrifugation (190g for 5 minutes).
6. Resuspend in 1 ml of serum-containing medium and transfer to a 24-well plate or to a 10 ml cell culture tube.
7. Analyze the cells immediately or after an incubation period. Delivery can be analyzed by assessing protein activity or visualizing intracellular fluorescence.

## Contact our Technical Assistance and Scientific Advice Service

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## Troubleshooting

Observations	Actions
Low efficiency of protein or antibody delivery	For adherent cells, ensure that they are 70-80% confluent on the day of the experiment.
	Optimize the amount of protein delivered (0.5 to 2 µg).
	Include an additional washing step with PBS to ensure that all traces of serum have been removed.
	Use protein as pure as possible.
	Optimize the ratio PULSin™/protein starting from 1 µl to 4 µl of PULSin™ per µg of protein.
	Perform a positive control delivery experiment with 1 µg of the control protein on your cell type (R-phycoerythrin, included in each PULSin™ kit).
	Ensure that the complexes are prepared in serum-free medium.
	If the cells can withstand it, centrifuge the culture plates for 5 min at 190g.
When using commercial antibodies, check the BSA concentration of the antibody and remove it when possible.	
Presence of aggregates	Ensure that cells are more confluent on the day of delivery, ideally 80%.
Cellular toxicity	Reduce the amount of protein used in the assay.
	Check protein concentration and ensure that the ratio PULSin™/protein is less or equal to 4 µl of PULSin™ for 1 µg of protein.
	Reduce the incubation time of the PULSin™/protein complexes with the cells from 4 h to 2 h. For very sensitive cells, include a centrifugation step described in section 1.4.