PROTOCOL

PULSin®
in vitro protein, antibody, and peptide transfection reagent

DESCRIPTION

PULSin® is a powerful reagent dedicated to the delivery 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. 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.

1. Transfection 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. Refer to Table 1 for protein delivery in other culture formats. Since the efficiency of protein delivery partly depends on cell confluency, the culture density should be optimized for each cell type.

Table 1. Recommended number of cells to seed the day before transfection with PULSin®.

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (mL)
96-well	10 000 - 15 000	0.3	0.2
24-well	70 000 - 100 000	1.9	1
12-well	100 000 - 180 000	3.8	2
6-well / 35 mm	200 000 - 300 000	9.4	3
60 mm / flask 25 cm^2	300 000 - 800 000	25 - 28	5
100 mm / flask 75 cm^2	1.10^6 - 2.10^6	75 - 78.5	10

1.2. Transfection protocol

The following protocol is given per well of a 24-well plate, 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.

For other culture format, please refer to Table 2. Guidelines for optimization are given in section 1.4.

1. Dilute 1 µg of protein in 100 µL of 20 mM HEPES in a microcentrifuge tube. Vortex gently and spin down briefly.
2. Vortex PULSin® reagent for 5 sec and spin down before use.
3. Add 4 µL of PULSin®. Vortex immediately and spin down briefly.
4. Incubate for 15 minutes at room temperature.
5. Wash cells once with 1X PBS or culture medium without serum. The washing step is critical to remove all traces of serum.
6. Add 900 µL of culture medium without serum per well.
7. Add 100 µL of protein/PULSin® mix per well and homogenize by gently swirling the plate.
8. After 4 hours of incubation at 37°C, remove the medium containing the protein/PULSin® complexes and replace with complete growth medium. Return the plate to the incubator.
9. Analyze protein activity or visualize intracellular fluorescence immediately or after an incubation period.

N.B.: For R-PE, the excitation by a laser at 488 nm induces a maximal light emission at 575 nm, and the optimal time of visualization is 16 hours after protein delivery.

Table 2. Protein delivery guidelines according to the cell culture vessel.

Culture vessel	Amount of protein (µg)	Volume of 20 mM HEPES Buffer (µL)	Volume of PULSin® reagent (µL)
96-well	0.3	20	1.2
24-well	1	100	4
12-well	2	150	8
6-well / 35 mm	4	200	16
60 mm / flask 25 cm^2	7	400	28
100 mm / flask 75 cm^2	10	1000	40

1.3. Transfection protocol for sensitive cells

Sensitive cells may not withstand the absence of serum for 4 hours; thus, we suggest an alternative protocol below. Proceed with Steps 1 to 6 as in 1.2, then add a short centrifugation step as indicated below.

1. Gently centrifuge the tissue culture plate for 5 min at 190 g (if the cells can withstand it) directly after adding the protein/PULSin® complexes onto the cells.
2. Incubate for 30 min at 37°C. Remove the medium containing the protein/PULSin® complexes and replace with complete growth medium. Return the plate to the incubator.

Analyze protein activity or visualize intracellular fluorescence immediately or after an incubation period.

1.4. Optimization guidelines

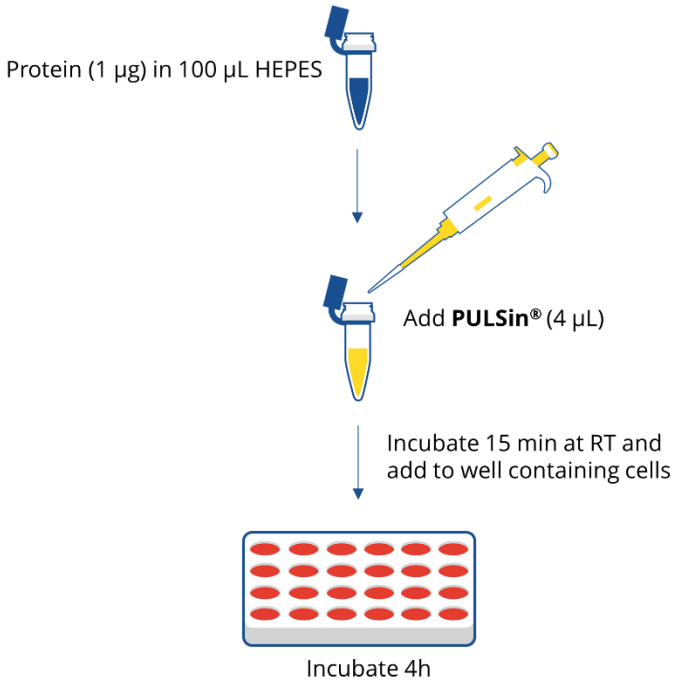
Optimization is highly recommended to obtain as high protein delivery efficiency as possible with PULSin®. In addition, variations may be observed from one cell line to another, even with the same protein. Thus, we recommend testing a range from 0.5 µg to 4 µg of protein, antibody, or peptide and 1 µL to 4 µL of PULSin® per well of 24-well plate (cf. Table 3).

Table 3. Optimization guidelines for PULSin®-mediated protein transfection (per well of 24-well plate).

Amount of protein (µg)	Volume of PULSin® reagent (µL)
0.5	1
	2
	4
2	1
	2
	4
4	1
	2
	4

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

Protein delivery protocol for a well of 24-well plates



2. Transfection protocol for suspension cells

2.1. Cell seeding

For protein delivery in suspension cells (Jurkat, THP-1, K-562, etc.), cells are counted and collected by centrifugation (190 g for 5 minutes) on the day of the experiment. Per well of a 24-well plate, seed $5x10^5$ cells in 1 mL of Opti-MEM® (without serum but containing glutamine). The following protocol is given as a starting point, for optimization please refer to section 1.4.

2.2. Protein tranfection protocol

100 µL of protein/PULSin® complexes are required per well of 24-well plate and are prepared as follows:

1. Dilute 1 - 2 µg of protein into 100 µL of 20 mM HEPES. Vortex gently and spin down briefly.
2. Vortex PULSin® reagent for 5 sec and spin down before use.
3. Add 2 - 4 µL of PULSin® into each tube. Vortex immediately and spin down briefly.
4. Incubate for 15 minutes at room temperature and add the protein/PULSin® mix into the 1 mL of cells at the density of $5x10^5$ cells/mL.
5. After 4 hours of incubation at 37°C, centrifuge the cells 5 min at 190 g and resuspend them in 1 mL complete growth medium. Return the plate to the incubator.
6. Analyze protein activity or visualize intracellular fluorescence immediately or after an incubation period

3. Troubleshooting

Observations	Actions
Low protein delivery efficiency	• Ensure that adherent cells are 70-80% confluent on the day of the experiment. • Optimize the amount of protein delivered (0.5 to 2 µg per well of 24-well plate). • 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 protein/PULSin® ratio from 1:1 to 1:4. • Perform a positive control delivery experiment with the positive control protein R-phycoerythrin (included in each PULSin® kit), using 1 µg per well of 24-well plate on your cells. • Ensure that the complexes are prepared in HEPES buffer and added to the cells in serum-free medium. • If the cells can withstand it, centrifuge the culture plates for 5 min at 190 g right after adding the protein/PULSin® complexes to the cells. • 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 protein/PULSin® ratio is lower than 1:4. • Reduce the incubation time of the protein/PULSin® complexes with the cells from 4 h to 2 h. For very sensitive cells, include a centrifugation step (5 min at 190 g right after adding the protein/PULSin® complexes to the cells) and incubate only 30 min as described in section 1.4.

4. Product Information

4.1. Ordering Information

Part N°	PULSin® Reagent	Number of transfection experiments
101000010	0.4 mL	25 delivery experiments in 6-well plate

4.2. Content

- 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.
- 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 maximal light emission at 575 nm.
- HEPES Buffer (20 mM), 20 mL (Part # 101000010) for protein dilution.

4.3. Reagent use and Limitations

For research use only. Not for use in humans.

4.4. Quality control

Every batch of PULSin® is tested by delivering R-phycoerythrin into HeLa cells. Certificates of Analysis are available online in your Customer Area: https://myaccount.polyplus-transfection.com/wp-login.php

4.5. Formulation and Storage

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

Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution, and customer support.

4.6. Trademarks

Polyplus-transfection® and PULSin® are registered trademarks of Polyplus-transfection S.A.

How to cite us: “PULSin® (Polyplus-transfection S.A, Illkirch, France)”.

4.7. Contact information

Do you have any technical question regarding your product?

- Website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87

Contact the friendly Scientific Support team which is composed of highly educated scientists, PhDs and Engineers, with extensive hands-on experience in cell culture and transfection. The Scientific Support is dedicated to help our customers reach their goals by proposing different services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc.

For any administrative question, feel free to contact our administration sales team:

- Reception Phone: +33 3 90 40 61 80
- Fax: +33 3 90 40 61 81
- Addresses:

Polyplus-transfection®		
Vectura 75, rue Marguerite Perey 67400 Illkirch France	1251 Ave of the Americas 34th fl. New-York - NY 10020 United States	Room 1506, Tower B, Sunyoung Center No. 28 Xuanhua Road Changning District, Shanghai China

Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.