Cell-IN

enhance cellular uptake

PROTOCOL

DESCRIPTION

Cell-IN is a loading reagent based on a polymer formulation manufactured at the Institute of Physical Chemistry Polish Academy of Sciences. Cell-IN ensures effective and cellular uptake of different compounds. Cell-IN is efficient on a wide variety of cell lines.

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1. LOADING PROTOCOL FOR 2D CELL CULTURE

1.1 Cell seeding

For optimal compound loading conditions, we recommend using cells that are 70 to 80% confluent at the time of loading. Typically, for experiments in 8-well plates, 30 000 cells* are seeded per well in 300 µL of cell growth medium 24h before loading. For other culture formats, refer to Table 1.

*highly proliferating cells, doubling time ~24h

Table 1. Recommended number of cells to seed the day before transfection examples.

Culture vessel*	Number of cells to seed	Surface area per well (cm^2)	The volume of medium per well to seed the cells (mL)
96-well	8 000 - 10 000	0.3	0.1
24-well	60 000 - 80 000	1.9	0.5
12-well	80 000 - 140 000	3.8	1
8-well	10 000 – 30 000	0.9	0.3
6-well / 35 mm	150 000 - 250 000	9.4	2
60 mm	250 000 - 800 000	21	5

*for other vessels contact us

1.2 Medium preparation

Before compound loading, two media hypertonic and hypotonic are needed to be prepared.

a. Hypertonic medium

I. Dissolve Cell-IN in serum-free medium in amount according to Table 2

Table 2. The recommended amount of medium for dissolving Cell-IN.

Number	Name	The volume of medium (mL)
1	Cell-IN Basic 0.1	0.1
2	Cell-IN Basic 0.5	0.5
3	Cell-IN Basic 1	1
4	Cell-IN Basic 5	5

II. If possible use an ultrasonic bath (36°C) for 30 minutes to enhance the effectiveness of the dissolving process

b. Hypotonic medium

I. Mix serum-free low glucose medium with sterile water at a rate of 6:4, e.g. 6 mL medium and 4 mL water

1.3 Loading protocol

I. Prewarm hypotonic medium and culture medium
II. Prepare working solution by mixing hypertonic medium with your stock solution in a ratio of 9:1, e.g. 18 µL hypertonic medium and 2 µL stock solution*
III. Remove medium from cells

IV. Add working solution in amount according to Table 3

*Please note that to obtain good efficiency the concentration of stock solution should be no less than 100 µM.

Table 3. The recommended amount of working solution for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of working solution per well (µL)
96-well	0.3	4
24-well	1.9	24
12-well	3.8	48
8-well	0.9	12
6-well / 35 mm	9.4	125
60 mm	21	280

*for other vessels, please contact us

V. Incubate for exact 10 minutes in 37˚C

VI. Remove working solution

VII. Add hypotonic medium in amount according to Table 4

Table 4. The recommended amount of hypotonic medium for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of hypotonic medium per well (µL)
96-well	0.3	90
24-well	1.9	460
12-well	3.8	900
8-well	0.9	280
6-well / 35 mm	9.4	1 800
60 mm	21	4 600

* for other vessels, please contact us

VIII. Incubate for exact 1.5 minutes in 37˚C

CAUTION! Incubation time should not exceed 2 minutes, due to possible cell swelling

IX. Remove hypotonic medium.

X. Add culture medium in amount according to Table 5.

Table 5. The recommended amount of culture medium for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of culture medium per well (µL)
96-well	0.3	100
24-well	1.9	500
12-well	3.8	1 000
8-well	0.9	300
6-well / 35 mm	9.4	2 000
60 mm	21	5 000

* for other vessels, please contact us

XI. Incubate for at least 10 minutes at 37˚C.

XII. Change culture medium to medium in which measurements are conducted

2. LOADING PROTOCOL FOR CELLS IN SUSPENSION

2.1 Cell seeding

For optimal compound loading conditions, we recommend using cells that are 70 to 80% confluent at the time of loading.

2.2 Medium preparation

Before compound loading, two media: hypertonic and hypotonic are needed to be prepared.

a. Hypertonic medium

I. Dissolve Cell-IN in serum-free medium in amount according to Table 6

Table 6. The recommended amount of medium for dissolving Cell-IN.

Number	Name	The volume of medium (mL)
1	Cell-IN Basic 0.1	0.1
2	Cell-IN Basic 0.5	0.5
3	Cell-IN Basic 1	1
4	Cell-IN Basic 5	5

II. If possible use an ultrasonic bath (36°C) for 30 minutes to enhance the effectiveness of the dissolving process

b. Hypotonic medium

I. Mix serum-free low glucose medium with sterile water at a rate of 6:4, e.g. 6 mL medium and 4 mL water

2.3 Loading protocol

I. Prewarm hypotonic medium and culture medium

II. Prepare working solution by combining hypertonic medium with your stock solution at a rate of 9:1, e.g. 18 µL hypertonic medium and 2 µL stock solution

III. Transfer 1 mL of medium with cells into a centrifuge tube

IV. Centrifuge medium with cells for 1 minute 2 500 rpm and remove the supernatant

V. Add 20 µL of working solution

VI. Incubate for exact 10 minutes in 37˚C

VII. Add 1 mL of hypotonic medium

VIII. Incubate for exact 1 minute in 37˚C

IX. Centrifuge hypotonic medium for 1 minute 2 500 rpm and remove the supernatant

X. Add 1 mL of culture medium

XI. Incubate for at least 10 minutes in 37˚C

XII. Transfer cells into culture vessel and add medium in which measurements are conducted

3. TRANSFECTION PROTOCOL

3.1 Cell seeding

For optimal compound transfection conditions, we recommend using cells that are 50 to 60% confluent at the time of transfection. Typically, for experiments in 8-well plates, 30 000 cells are seeded per well in 300 µL of cell growth medium 24h before transfection and 48h before experiments. For other culture formats, refer to Table 7.

Table 7. Recommended number of cells to seed the day before transfection examples.

Culture vessel	Number of cells to seed	Surface area per well (cm^2)	The volume of medium per well to seed the cells (mL)
96-well	8 000 - 10 000	0.3	0.1
24-well	60 000 - 80 000	1.9	0.5
12-well	80 000 - 140 000	3.8	1
8-well	10 000 – 30 000	0.9	0.3
6-well / 35 mm	150 000 - 250 000	9.4	2
60 mm	250 000 - 800 000	21	5

* for other vessels, please contact us

3.2 Medium preparation

Before compound transfection, two media: hypertonic and hypotonic are needed to be prepared.

a. Hypertonic medium

I. Dissolve Cell-IN in serum-free medium in amount according to Table 8

Table 8. The recommended amount of medium for dissolving Cell-IN.

Number	Name	The volume of medium (mL)
1	Cell-IN Basic 0.1	0.1
2	Cell-IN Basic 0.5	0.5
3	Cell-IN Basic 1	1
4	Cell-IN Basic 5	5

II. If possible use an ultrasonic bath (36°C) for 30 minutes to enhance the effectiveness of the dissolving process

b. Hypotonic medium

I. Mix serum-free low glucose medium with sterile water at a rate of 6:4, e.g. 6 mL medium and 4 mL water

3.3 Transfection protocol

I. Prewarm hypotonic medium and culture medium
II. Prepare working solution by mixing hypertonic medium with 125-250 ng/µl* of desired nucleic acid (NA) in a ratio of 9:1, e.g. 18 µL hypertonic medium and 2 µL NA

III. Centrifuge working solution in a vortex centrifuge
IV. Incubate the working solution at room temperature for 5 min
V. Remove medium from cells
VI. Add working solution in amount according to Table 9
*Please note, that the appropriate concentration of the compound strongly depends on many parameters, such as the size of DNA to be loaded. For the first time, we recommend trying a concentration of 200 ng/µl.

Table 9. The recommended amount of working solution for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of working solution per well (µL)
96-well	0.3	4
24-well	1.9	24
12-well	3.8	48
8-well	0.9	12
6-well / 35 mm	9.4	125
60 mm	21	280

* for other vessels, please contact us

VII. Incubate for exact 10 minutes in 37˚C
VIII. Remove working solution
IX. Add hypotonic medium in amount according to Table 10

Table 10. The recommended amount of hypotonic medium for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of hypotonic medium per well (µL)
96-well	0.3	90
24-well	1.9	460
12-well	3.8	900
8-well	0.9	280
6-well / 35 mm	9.4	1 800
60 mm	21	4 600

* for other vessels, please contact us

X. Incubate for exact 1.5 minutes in 37˚C
CAUTION! Incubation time should not exceed 2 minutes, due to possible cell swelling
XI. Remove hypotonic medium
XII. Add culture medium in amount according to Table 12

Table 12. The recommended amount of culture medium for the different vessels.

Culture vessel	Surface area per well (cm²)	The volume of culture medium per well (µL)
96-well	0.3	100
24-well	1.9	500
12-well	3.8	1 000
8-well	0.9	300
6-well / 35 mm	9.4	2 000
60 mm	21	5 000

* for other vessels, please contact us

XIII. Gene expression is detectable after 24h and increases with time

4. TROUBLESHOOTING

Observations	Actions
Low efficiency	Use compound with an initial higher concentration
Too high efficiency	Use compound with an initial lower concentration

5. PRODUCT INFORMATION

5.1 Ordering information

Number	Name	Mass of powder (mg)
1	Cell-IN Basic 0.1	11.6
2	Cell-IN Basic 0.5	58
3	Cell-IN Basic 1	116
4	Cell-IN Basic 5	580

5.2 Content

Cell-IN Basic 5 reagent is sufficient to perform up to 50 loadings in 8-well plates or 6 loadings in 6-well plates.

5.3 Reagent use and Limitations

For research use only. Not for use in humans.

5.4 Quality control

Every batch of Cell-IN reagent is tested by loading TRITC-dextran 75 kDa into HeLa cells.

5.5 Formulation and Storage

Cell-IN should be stored at 20°C and is stable for at least one year when stored appropriately. However, dissolved Cell-IN in a serum-free medium can also be stored at 4°C and is stable no longer than one month.

5.6 Trademarks

How to cite us: “Cell-IN (IPC PAS, Warsaw, Poland)”