

I. MEDIUM PREPARATION

Hypertonic medium:

Dissolve Cell-IN in serum-free medium in amount according to **Table 1**.

If possible, use an ultrasonic bath (36°C) for 30 minutes to enhance the effectiveness

Hypotonic medium:

Mix serum-free low glucose medium with sterile water at the rate of 6:4



II. LOADING PROTOCOL

a. Prewarm hypotonic medium and culture medium

b. Prepare working solution by mixing hypertonic medium with the desired compound in the ratio of 9:1

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



c. Remove medium from cells

d. Add working solution in amount according to **Table 2**.



e. Incubate for exact 10 minutes in 37°C



f. Remove working solution

g. Add hypotonic medium in amount according to **Table 3**.



h. Incubate for exact 2 minutes in 37°C



i. Remove hypotonic medium

j. Add culture medium in amount according to **Table 4**.



k. Incubate for at least 10 minutes in 37°C



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

Table 1. The recommended amount of medium for dissolving Cell-IN.

<i>Number</i>	<i>Name</i>	<i>The volume of medium (mL)</i>
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

Table 2. The recommended amount of working solution for different vessel.

<i>Culture vessel</i>	<i>Surface area per well (cm²)</i>	<i>The volume of working solution per well (μL)</i>
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

Table 3. The recommended amount of hypotonic medium for different vessel.

<i>Culture vessel</i>	<i>Surface area per well (cm²)</i>	<i>The volume of hypotonic medium per well (μL)</i>
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

Table 4. The recommended amount of culture medium for different vessel.

<i>Culture vessel</i>	<i>Surface area per well (cm²)</i>	<i>The volume of culture medium per well (μL)</i>
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