Cellplus Bio

MetaCell® PEI 40K

Product Description

MetaCell® PEI 40K (1mg/mL) is a high-charge cationic polymer with linear polyethyleneimine as its main component, having a molecular weight of 40,000. It is positively charged and can effectively bind with negatively charged nucleic acids to form complexes, which are then imported into cells. It is suitable for transfection of plasmid DNA into cells. MetaCell® PEI 40K has been widely verified to be applicable to various cell lines, including HEK293, HEK293T, CHO-K1, COS-1, COS-7, NIH/3T3, Sf9, HepG2, and HeLa cells, among others.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Product Name	Cat No.	Size	Storage	Shelf Life	Application
MetaCell® PEI 40K	L5001-0010	10mL	2-8°C, protected from light	12 months	Efficient transient transfection of CHO & HEK293 cells
	L5001-0100	100mL			

Reagent Preparation

Dilution Solution Preparation: Prepare 150 mM NaCl using cell culture-grade water (weigh 876.6 mg of high-purity NaCl and add it to 80 mL of cell culture-grade water, dissolve completely, then adjust the volume to 100 mL, and filter through a 0.1 or 0.2 μm filter to sterilize).

Precautions

1. Please ensure to use high-quality endotoxin-free plasmid DNA. Determine the DNA concentration by measuring the absorbance at 260 nm, and confirm the purity of the DNA with the ratio of absorbance at 260 nm to 280 nm (the ratio should be within the range of 1.8 to 2.0). If possible, check the integrity of the plasmid by agarose gel electrophoresis.
2. Use healthy cells that are properly stored and frequently passaged. Ensure that the culture medium is free from bacterial, fungal, or mycoplasmal contamination. If the cells are recently thawed cryopreserved cells, passage at least twice before transfection.
3. For certain types of cells such as HEK-293, HEK-293T, NIH/3T3, and COS cells, plating two days before transfection can significantly increase the expression level of recombinant proteins. If choosing to plate two days before transfection, reduce the plating density to ensure that the cell confluence is still 60-80% at the time of transfection.
4. For cells sensitive to contact inhibition, reduce the plating density appropriately.
5. For safety and health, please wear a lab coat and disposable gloves during the procedure.

Operation Instructions — Adherent Cells

- Plating

Plate the cells 18-24 hours before transfection, adjusting the appropriate cell density (refer to Table 1) to achieve 60-80% confluence at the time of transfection.

Note: High serum levels can inhibit transfection efficiency. In most cases, low serum levels (≤5%) yield the highest transfection efficiency.

- Transfection Steps (for a single well of a 6-well plate)

1. Replace the medium in each well with 3 mL of fresh growth medium containing 2% serum 1-2 hours before transfection.
2. Prepare the PEI 40K-DNA transfection complex (follow the order strictly):
   (1) Add 2 μg of plasmid DNA to 300 μL of dilution buffer and mix gently by low-speed vortexing.
   (2) Add 8 μL of MetaCell® PEI 40K (1 mg/mL) (DNA/PEI 40K = 1:4) to the mixture and vortex gently for 5 seconds.
   (3) Incubate the mixture at room temperature in a sterile environment for 20 minutes to form the PEI 40K-DNA transfection complex.
   (4) Gently mix by pipetting up and down three times.
3. Transfer the PEI 40K-DNA transfection complex into the well. Gently rock the culture dish or vortex slightly to distribute the complex evenly.

Note: The above steps can be scaled up or down by adjusting the volume of the PEI 40K-DNA complex in the dilution buffer (dilution buffer is 10% of the total culture volume), ensuring a DNA/PEI 40K ratio of 1:4 (refer to Table 2).

Table1. Recommended Inoculation Densities for Different Culture Vessels

Culture Vessels	Surface Area(cm^2)	Cell Seeding Density	Culture Vessels	Surface Area(cm^2)	Cell Seeding Density
96-well plate	0.3	$1.2\text{-}2.4\times10^4$	35mm culture dish	9.6	$3.5\text{-}7.0\times10^5$
48-well plate	1.0	$4.0\text{-}8.0\times10^4$	60mm culture dish	21	$0.9\text{-}1.8\times10^6$
24-well plate	1.9	$0.8\text{-}1.6\times10^4$	100mm culture dish	58	$2.2\text{-}4.4\times10^6$
12-well plate	3.5	$1.5\text{-}3.0\times10^4$	T75 flask	75	$3.0\text{-}6.0\times10^6$
6-well plate	9.6	$4.0\text{-}8.0\times10^4$	250mL shake flask	175	$0.7\text{-}1.4\times10^7$

Table2. Recommended Dosages of Each Component for Transfection in Various Culture Systems

Culture Vessels	Culture Volume	Plasmid DNA (μg)	Dilution Buffer (mL)	PEI 40K (μL)
6-well single plate	3	2-4	0.3	8-16
35mm culture dish	3	2-4	0.3	8-16
60mm culture dish	5	6-12	0.5	24-48
100mm culture dish	10	12-24	1.0	48-96
T75 flask	15	18-36	1.5	72-144
250mL shake flask	50	50-100	2.5	200-400

- Incubation

1. Culture cells in a 37°C incubator with 5% CO_2 for 12-18 hours after transfection. Remove the medium containing the PEI 40K-DNA complex and replace with fresh growth medium.
2. Typically, recombinant protein expression can be detected 36-48 hours post-transfection. The maximum level of expression is generally observed 72-96 hours post-transfection.

Operation Instructions — Suspension Cells (using CHO cells as an example)

Recommendation	Amount of DNA	Volume of PEI	Volume of MetaCell® Media for complexes preparation
Range	0.8-1.5 μg	7-12 μL	0.08 mL
Scenario 1	1.5 μg	10 μL	0.08 mL
Scenario 2	2.0 μg	12 μL	
Scenario 3	0.8 μg	7 μL	0.08 mL
Scenario 4	1.5 μg	10 μL	

Note: Actual usage amounts should be optimized based on the cell line and medium, among other factors, to refine the process.