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Version: 2.0.510.1

TransPi™ Mate DNA Transfection Reagent

Protocol for Plasmid DNA Transfection in Adherent Cells

Product Description

TransPi™ Mate is a novel cationic polymer–based transfection reagent composed of a hyperbranched poly(β-amino ester) (HPAE). When mixed with plasmid DNA in an aqueous solution, it can assemble and complex with negatively charged pDNA through electrostatic interactions, hydrogen bonds, and other forces. Without the need for incubation, uniform-sized transfection complexes (nanoparticles) with a positively charged surface are formed, and these complexes can remain stable for more than 3 hours. Benefiting from its optimized structural design, TransPi™ Mate can condense plasmids more effectively, resulting in nanoparticles with higher uniformity and stability. Meanwhile, it enables high cellular uptake, efficient endosomal escape and intracellular release, and can be completely hydrolyzed and degraded eventually. As a result, it exhibits superior transfection efficiency and biocompatibility.

Components and Storage Conditions

1．TransPi™ Mate reagent

−20 °C (freeze); aliquot if needed to reduce freeze–thaw cycles. Supplied at 100 mg/mL in 100 % DMSO. In the final transfection mixture, the DMSO content is ≈ 0.1 %, which has no effect on cell viability.

In the standard operating procedure, 200 µL of TransPi™ Mate is sufficient for 800 transfections in 24-well plates (μg_{pDNA}=1.25 µg， $\mu L_{reagent}$: μg_{pDNA}=0.2: 1).

2．Buffer for TransPi™ Mixing

0.22 µm-filtered; store 4 °C or −20 °C. Acidic diluent buffer; for sensitive cells it can be replaced with PBS/DPBS or serum-free media (e.g. Opti-MEM).

Catalog No.	TransPi™ Mate（mL）	Mixing Buffer（mL）
1000M-0.1	0.1	20
1000M-0.5	0.5	100
1000M-1	1	200

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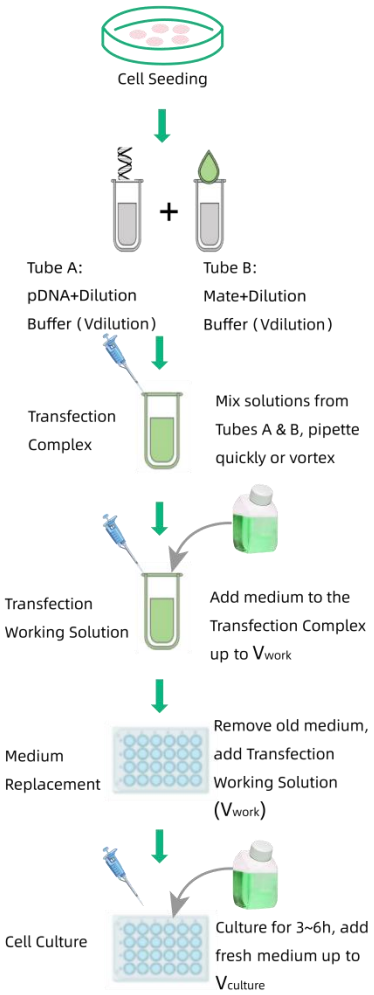
Experimental Procedure

1. Cell Seeding

To achieve optimal transfection efficiency in adherent cells using TransPi™ Mate, seed the cells one day before transfection so that they reach 60–80% confluency on the day of transfection. For recommended seeding densities of HEK293T cells in different culture formats, refer to Table 1. Seed the cells according to the suggested numbers so that the confluency at the time of transfection is approximately 60–80%, and adjust as needed based on experimental experience.

Table 1. Recommended Cell Seeding Density Before Transfection

Culture Format	Recommended Cell Number (cells/well)	Growth Surface Area (cm^2)	Recommended Culture Volume $V_{culture}$(mL)
Culture dish			
35 mm	$5.5\times10^5 \sim 8.0\times10^5$	8.8	2
60 mm	$1.3\times10^6 \sim 2.0\times10^6$	21.5	5
100 mm	$3.5\times10^6 \sim 5.5\times10^6$	56.7	12
150 mm	$9.0\times10^6 \sim 1.5\times10^7$	145	30
Culture plate			
6-well	$6.0\times10^5 \sim 9.0\times10^5$	9.6	2
12-well	$2.0\times10^5 \sim 3.0\times10^5$	3.5	1
24-well	$1.0\times10^5 \sim 1.5\times10^5$	1.9	0.5
48-well	$6.0\times10^4 \sim 1.0\times10^5$	1.1	0.2
96-well	$2.0\times10^4 \sim 3.0\times10^4$	0.32	0.1
Culture flask			
T-25	$1.5\times10^6 \sim 2.5\times10^6$	25	5
T-75	$5.0\times10^6 \sim 7.0\times10^6$	75	15
T-175	$1.0\times10^7 \sim 1.6\times10^7$	175	35
T-225	$1.5\times10^7 \sim 2.0\times10^7$	225	45



2. DNA Transfection Procedure

The following procedure describes transfection in HEK293T cells using a total of 1.25 μg pDNA per well in a 24-well plate. For other culture formats, please refer to Table 2.

1. Remove the pDNA, TransPi™ Mate transfection reagent, and the Buffer for TransPi™ Mixing (which can be replaced with PBS/DPBS or serum-free medium) from the refrigerator and allow them to reach room temperature. Wipe off surface condensation carefully and strictly avoid the introduction of water, which can cause

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reagent hydrolysis and degradation. Warm the cell culture medium in a 37 °C water bath.

2. Prepare two 1.5 mL microcentrifuge tubes labeled A and B. Dilute the pDNA (tube A) and TransPi™ Mate reagent (tube B) separately with the Buffer for TransPi™ Mixing to the total volume （$V_{dilution}$）, and pipette up and down 5–10 times to mix.

3. Add the entire solution from tube B into tube A at once. Rapidly pipette 15–20 times or vortex for 3–5 seconds to mix thoroughly and form transfection complexes.

Note 1: The order of mixing can be reversed. **Do not invert the tubes slowly up and down;** ensure that the liquids in Tubes A and B are mixed quickly! Bubbles will not affect transfection efficiency.
Note 2: For sensitive cells, PBS/DPBS or serum-free medium may be used instead of the provided dilution buffer for testing.
Note 3: The transfection complexes can be used immediately without incubation and remain stable and effective at room temperature for at least 3 hours.

4. Add 225 µL of fresh culture medium to the 25 µL transfection complex obtained above to form the working transfection solution (V_{work} = 250 µL). Pipette 3–5 times to mix thoroughly.

5. Remove the cell culture plate from the incubator, discard the old medium, and add the prepared transfection working solution to the designated wells. Return the plate to the incubator for transfection.

6. After incubation at 37 °C for 3–6 hours, remove the plate and add 250 µL of fresh medium to each well to bring the total culture volume to 500 µL ($V_{culture}$). The final proportion of the transfection complex in the culture (5–10 %) is acceptable, and no medium change is required.

7. Proceed with subsequent harvesting and analysis as required by the experiment.

3. Parameter Calculation

First determine the culture volume for the chosen format ($V_{culture}$). For the first 3–6 h of transfection, set the working volume V_{work} = ½ $V_{culture}$ (the coefficient may be further reduced provided cell status is not affected). Maintain the plasmid concentration at the cell surface (C_{pDNA} in V_{work}) at ≥ 5 µg/mL. Use the transfection reagent at a ratio of $\mu L_{reagent}$: μg_{pDNA} = 0.2. The complex volume may account for 5–10% of V_{work}. Table 2 uses 10% as an example for calculation. You may adjust the transfection parameters individually following this logic; standard parameters for common culture formats are provided in Table 2.

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Table 2. Standard Transfection Parameters for Different Culture Formats

Culture Formate	μg pDNA	μL reagent	$V_{dilution}$ (μL)	V_{work} (mL)	Add fresh medium after 3-6h (mL)	Parameters used for calculation (customizable)		
						$V_{culture}$ (mL)	C_{pDNA} in V_{work} (μg/mL)	$μL_{reagent}$: $μg_{pDNA}$
Culture dish								
35 mm	5	1	50	1	1	2	5	0.2
60 mm	12.5	2.5	125	2.5	2.5	5	5	0.2
100 mm	30	6	300	6	6	12	5	0.2
150 mm	75	15	750	15	15	30	5	0.2
Culture plate								
6-well	5	1	50	1	1	2	5	0.2
12-well	2	0.4	20	0.4	0.6	1	5	0.2
24-well	1.25	0.25	12.5	0.25	0.25	0.5	5	0.2
48-well	0.5	0.1	5	0.1	0.1	0.2	5	0.2
96-well	0.25	0.05	2.5	0.05	0.05	0.1	5	0.2
Culture flask								
T-25	12.5	2	125	2.5	2.5	5	5	0.2
T-75	37.5	7.5	375	7.5	7.5	15	5	0.2
T-175	87.5	17.5	875	17.5	17.5	35	5	0.2
T-225	112.5	22.5	1125	22.5	22.5	45	5	0.2

Optimization Recommendations

You can optimize transfection performance by adjusting the amounts of TransPi™ reagent, pDNA, and the reagent-to-DNA ratio ($μL_{reagent}$: $μg_{pDNA}$). For 24-well plates, we recommend testing the conditions listed in Table 3. For other culture formats, the corresponding parameters can be calculated using the provided calculation sheet.

(1) To save plasmid and reagent:

During the first 3–6 hours after transfection, proportionally reduce V_{work}, the complex volume, and the amounts of pDNA and reagent.

(2) To enhance transfection efficiency:

Under the same conditions, increase the reagent-to-DNA ratio to 0.22–0.30 μL/μg; or during the first 3–6 hours after transfection, further raise CpDNA in Vwork to 5–10 μg/mL or higher (this can be achieved by reducing the culture volume Vwork or increasing the plasmid amount; lowering Vwork also helps reduce cytotoxicity).

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Table 3. Recommended Test Parameters for Cell Transfection in 24-Well Plates

Group	V_{work} (µL)	C_{pDNA} in V_{work} (µg/ml)	$\mu L_{reagent}$: μg_{pDNA}	µg pDNA	µL reagent	$2*V_{dilution}$ (µL)	V_{work}-$2*V_{dilution}$ (µL)
Baseline	250	5	0.2	1.25	0.25	25	225
High Reagent Does	250	5	**0.25**	**1.25**	**0.31**	25	225
Low Plasmid Dose	**188**	5	0.2	**0.94**	**0.188**	19	169

Common Problems and Solutions

Problem	Solution
Low transfection efficiency	1. Ensure that the cells used are between passages 2–20 after recovery, are not over-confluent, and are free from mycoplasma contamination. 2. Use high-quality pDNA with high supercoiling and no endotoxin contamination. 3. Optimize transfection parameters according to the optimization recommendations and Table 4
Poor cell condition or detachment after transfection	1. Ensure that the cells are not contaminated. 2. Verify that the plasmid is endotoxin-free. 3. Reduce C_{pDNA} in V_{work} to ≤ 4 µg/mL and/or lower the $\mu L_{reagent}$: μg_{pDNA} ratio to 0.2. 4. If the cells were not sufficiently confluent at the time of transfection, refer to the recommended seeding densities in Table 1 and perform transfection when cells reach higher confluency. Add or replace medium during later stages of culture as needed. 5. For viral packaging, if harvesting virus at 72 h, also collect once at 48 h and replenish with fresh medium before the second harvest at 72 h, to prevent cell stress or detachment due to nutrient depletion. 6. Pay attention to the cytotoxicity of the expressed protein. If the expressed protein is toxic to cells, reduce the plasmid amount.

Quality Control

Each batch of TransPi™ Mate is verified during production and final quality release by NMR and GPC to confirm polymer structure, molecular weight, and molecular weight distribution, ensuring batch-to-batch consistency within the acceptance criteria. In vitro transfection activity is further validated using HEK 293 cells.

Reagent Usage Restrictions

For research use only. Not for use in humans.

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