MaxPrep™ Transfection Reagent

For the transient and stable transfection in mammalian cells

CAT. NO. TR-518-0010, 1 ml

DESCRIPTION

MaxPrep™ is a proprietary polymer based transfection reagent formulated for the transfection of DNA into mammalian cells, particularly for large scale protein production (tested up to 1 L in suspension cell lines or attached cell lines).

- High efficiency but low toxicity in various cell types;
- MaxPrep™ /DNA complex can be added directly to cells in culture medium, in the presence of antibiotics and serum;
- Slow release formulation guaranteed the low toxicity and high transfection efficiency during the incubation;
- Eliminates the need for media changes after transfection;
- Excellent for industry level protein expression such as in suspension cells.

IMPORTANT GUIDELINES

- To achieve highest efficiency, transfect cells at high density (90-95% confluence is recommended);
- To minimize cytotoxicity, transfect cells in presence of serum (10%) and antibiotics;
- Optional (for adherent cells): change medium with serum (10%) antibiotics 5 hours post transfection.

Size

1 ml (sufficient for transfection of 333 µg DNA or 333 transfections in 24-well plates).

STORAGE

Store at -20°C for up to 2 years and 4 °C for 3 months from the date of shipment. MaxPrep™ is shipped at room temperature.

PROTOCOL FOR MAMMALIAN CELL TRANSFECTION

For Adherent Cells (24-well Plate)

1. **Cell Seeding**
   Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 90~95% confluence at the time of transfection. Freshly complete culture medium with serum and antibiotics is added to each well 30~60 minutes before transfection.

   Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some cell lines, higher transfection efficiencies are observed in the presence of serum and antibiotics. We recommend you use complete medium containing serum and antibiotics initially.

2. **Preparation of MaxPrep™-DNA Complex and Transfection Procedures (24-Well Plate)**
   For different cell types, the optimal ratio of MaxPrep™ (µl):DNA (µg) varies from 1:1 to 3:1. We recommend the MaxPrep™ (µl):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency

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with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and MaxPrep™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to Table 1 for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines are given in the standard protocol described below.

2.1. Add 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin briefly to bring drops to bottom of the tube.

2.2. Add 3 µl of MaxPrep™ reagent into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

2.3. Immediately add the diluted MaxPrep™ Reagent to the diluted DNA solution.
Important: do not mix the solutions in the reverse order !

2.4. Vortex the solution immediately. Spin down briefly to bring liquid drops to bottom of the tube.

2.5. Leave the solution **undisturbed** for 15-20 min at room temperature to allow MaxPrep™ -DNA complexes to form.
Note: Never keep the DNA-GEM complex longer than 20 minutes.

2.6. Add 100 µl MaxPrep™ -DNA complex drop-wise into each well containing cells and medium. Mix gently by rocking the plate back and forth.

2.7. Change medium 48 hours post transfection.
For sensitive cells, to lower cytotoxicity, remove MaxPrep™ **-DNA complex and replace with complete medium 5 or 24 hours after transfection.**

2.8. Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture Vessel.

Culture Dish	Amount of DNA (µg)	MaxPrep™ (µl)	MaxPrep™/DNA Incubation Volume (µl)	Final Culture Volume (ml)
96-well plate	0.2	0.6	20	0.2
48-well plate	0.5	1.5	40	0.5
24-well plate	1.0	3	100	0.8
12-well plate	1.5	4.5	200	1
6-well plate	3	6	300	2
35 mm dish	3	9	200	2
60 mm dish	5	15	500	3
100 mm dish	8	24	1000	6
T75 flask	18 – 36	54-108	1500	10

For Suspension Cells (6-Well Plate)

1. Cell Seeding

Suspension cells are typically seeded the day of the transfection at a density of 0.5~1.0 x 10^6 cells per ml of culture. For optimal transfection conditions with GEM, seed the number of cells adapted to the culture vessel format according to Table 2. -30~60 minutes before transfection, warm fresh medium in a 37 ^oC water bath. Aspirate out the old medium from each well and add 0.5 ml fresh medium with serum and antibiotics.

Table 2. Recommended Number of Suspension Cells to Seed

Culture Dishes	Number of Cells to Seed
100 mm Dish	5x 10^6 - 1x 10^7
60 mm Dish	2x 10^6 – 5x 10^6
35 mm Dish	5x 10^5 – 2x 10^6
6-well Plate	2x 10^5 – 5x 10^5
24-well Plate	1x 10^5 – 2x 10^5
48-well Plate	5x 10^4 – 1x 10^5
96-well Plate	2x 10^4 – 5x 10^4

2. MaxPrep™-DNA Complex Preparation and Transfection Procedures

For different cell types, the optimal ratio of MaxPrep™ (µl):DNA (µg) varies from 2:1 to 3:1. We recommend the MaxPrep™ (µl):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and MaxPrep™ reagent.

The following protocol is given for transfection in 6-well plate. The protocol can be scaled up or down according to culture volume.

2.1. For each well, dilute 2 µg of DNA into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.

2.2. For each well, dilute 6 µl of MaxPrep™ reagent into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.

2.3. Add the 100 µl MaxPrep™ solution immediately to the 100 µl DNA solution all at once.
Important: do not mix the solutions in the reverse order !

2.4. Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.

2.5. Incubate for 15~20 minutes at room temperature.

2.6. Add the 200 µl MaxPrep™ / DNA mixture drop-wise onto the serum-containing medium in each well, homogenize the mixture by gently swirling the plate.

2.7. Incubate at 37 ^oC and 5% CO_2 in a humidified atmosphere.

2.8. Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800 x g and then resuspended in the desired medium or buffer.

PROTOCOL FOR HADR-TO-TRANSFECT MAMMALIAN CELLS

Important Guidelines

- For high efficiency, transfect cells at high density. 90~95% confluence is highly recommended.
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.
- Change medium with serum.

1. Cell Culture

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluence at the day of transfection.

2. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect mammalian cells in 6-well plates, refer to **Table 3** for optimal cell number per well per culture vessels' surface area. The optimal transfection conditions for mammalian cells are given in the standard protocol described below.

2.1. Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.

Note: Cells that are difficult to detach may be placed at 37 °C for 5~15 min to facilitate detachment.

2.2. Centrifuge the required 1.2×10^6 cells per well for 6-well plate at 150xg at room temperature for 10 min.

2.3. Use fine tip pipette to remove supernatant completely so that no residual medium covers the cell pellet.

Table 3. Optimal Cell Number Per Well in Different Culture Vessels

Culture Dishes	Surface Area (cm^2)	Number of Cells to Seed
T75 Flask	75	9.6×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.2×10^6
6-well Plate	9.6	1.2×10^5
12-well Plate	3.5	4.4×10^5
24-well Plate	1.9	2.4×10^5
48-well Plate	1.0	1.1×10^5
96-well Plate	0.3	3.1×10^4

3. Preparation and Application of Transfection Complex

For hard-to-transfect mammalian cells, the optimal ratio of GEM (µl):DNA (µg) is 4:1.

To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GEM Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 4** for transfection in other culture formats.

3.1. For each well of 6-well plate, dilute 8 µl of GEM reagent into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

3.2. Add the diluted GEM Reagent immediately to the diluted DNA solution all at once.

3.3. Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10 minutes at room temperature to allow transfection complexes to form.

Important: Never keep the transfection complexes longer than 15 minutes.

3.4. Gently resuspend the cell pellet prepared from **Step 2** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.

3.5. At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate.

3.6. Remove transfection complex containing medium gently and refill with complete culture medium 5~12 hours after plating.

3.7. Check transfection efficiency 24 to 48 hours post transfection.

PROTOCOL FOR GENERATION OF LENTIVIRUS FROM 293T CELLS

Important Guidelines:

- For high efficiency, transfect cells at high density. 90~95% confluence is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics
- Change medium with serum (10%) and antibiotics 5 hours post transfection is optional

1. Cell Seeding (see Table 5):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~95% confluence at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

2. Preparation of GEM-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

2.1. Cell confluence should be ~95 % at the day of transfection.

2.2. For each 10 cm dish, add 5.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.

2.3. For each dish, dilute total 45 µg of DNA (three DNAs with 15 µg each) into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.

2.4. For each dish, dilute 60 µl of GEM reagent into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

2.5. Add the diluted GEM Reagent immediately to the diluted DNA solution all at once. (***Important: do not mix the solutions in the reverse order !)***

2.6. Vortex- mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15~20 minutes at room temperature to allow GEM-DNA complexes to form. **Note:** Never keep the DNA/GEM complex longer than 20 minutes.

2.7. Add the 1000 µl GEM / DNA complex dropwise onto the medium in each dish and homogenize the mixture by gently swirling the plate.

2.8. Remove DNA/GEM complex-containing medium and replace with fresh complete serum/antibiotics containing medium 5 hours post transfection.

2.9. Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

PROTOCOL FOR TRANSFECTING BACMIDS INTO SF9 CELLS

1. Count Sf9 cells, and adjust cell density to 5 x10^5 cells/ml in un-supplemented SF900II media.
2. Seed 2 ml of cell suspension per well (1 x 10^6 cells/well) in 6-well plate.
3. Label 2 wells as “negative control”, 2 wells as “1 µg DNA”, and 2 wells as “2 µg DNA”.
4. Incubate dishes at 27°C for 30-60 minutes (enough time to allow the cells to attach to the bottom of the wells).
5. Aliquot 500 µl of sterile diluent (150 mM NaCl) into three 1.5 ml Eppendorf tubes. Label the tubes “0”, “1 µg”, and “2 µg”. These will serve as 2.5X Master Mixes for each of the three conditions.

   NOTE: The sterile diluent should be 150 mM NaCl which is essential for DNA/GEM complex formation.

   IT IS IMPORTANT THAT THE DNA IS ADDED FIRST AND THE GEM Reagent IS ADDED SECOND TO EACH TUBE.
6. Aliquot 2.5 µg of bacmid into the “1 µg” Master Mix tube.
7. Aliquot 5 µg of bacmid into the “2 µg” Master Mix tube.
8. Briefly vortex the tubes.
9. To the “1 µg” Master Mix tube, add 10 µl of GEM and ***IMMEDIATELY VORTEX*** for 5 seconds.
10. To the “2 µg” Master Mix tube, add 20 µl of GEM Reagent and ***IMMEDIATELY VORTEX*** for 5 seconds.
11. Allow the Master Mix tubes to sit in the hood for 10~15 minutes.