MesGen™ Transfection Reagent

Cat # MTR2030 **Size #** 500ul & 1ml & 3ml

DESCRIPTION

MesGen™ Transfection Reagent is a novel molecule based on a polymer formulation manufactured at Polyplus-transfection. MesGen™ ensures effective and reproducible DNA transfection into mammalian cells. MesGen™ is extremely efficient on a wide variety of cell lines. This powerful reagent only requires low amounts of nucleic acid per transfection, hence resulting in very low cytotoxicity.

1 TRANSIENT

1.1. Cell Seeding

For optimal DNA transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 6-well plates, 200,000 cells are seeded per well in 2 ml of cell growth medium 24 h prior to transfection. For other culture formats, refer to Table 1.

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

Culture vessel	Number of adherent cells to seed	Surface area per well (cm2)	Volume of medium per well to seed the cells (ml)
96-well	7,500 – 10,000	0.3	0.1
24-well	50,000 – 80,000	1.9	0.5
12-well	80,000 – 150,000	3.8	1
6-well / 35 mm	150,000 – 250,000	9.4	2
60 mm / flask 25 cm2	250,000 – 800,000	25 - 28	5
100 mm / flask 75 cm2	1 x 10^6 - 2 x 10^6	75 - 78.5	10
150 mm / flask 175 cm2	2 x 10^6 - 5 x 10^6	153 - 175	20

The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 2.

1. Dilute 2 µg DNA into 200 µl serum-free medium. Mix by vortexing.
2. Add 1 - 2 µl Transfection Reagent, vortex for 10 s, spin down briefly.
3. Incubate for 30 min at RT.
4. Add 200 µl of transfection mix per well drop wise onto the cells in serum-free medium, and distribute evenly.
5. Gently rock the plates back and forth and from side to side.
6. Replace transfection medium after 4 h by cell growth medium and return the plates to the incubator.
7. Analyze after 24 h or later.

Table 2. DNA transfection guidelines according to the cell culture vessel per well.

Culture Vessel	Volume of serum-free medium (µl)	Amount of DNA (µg)	Volume of MesGen™ reagent (µl)	Volume of growth medium (ml)
96-well*	30	0.1	0.05 - 0.1	0.1
24-well	100	0.5	0.25- 0.5	0.5
12-well	100	1.0	0.5- 1.0	1
6-well / 35 mm	200	2.0	1.0 – 2.0	2
60 mm / flask 25 cm2	400	4.0	2.0 – 4.0	5
100 mm / flask 75 cm2	500	10.0	5.0 – 10.0	10
150 mm / flask 175 cm2	1000	20.0	10.0 – 20.0	20

* Prepare a master mix of minimum 50 µl to allow accurate pipetting and homogenous preparation of the complexes

2 TROUBLESHOOTING

Low DNA transfection efficiency

Action 1 : Optimize the volume of MesGen reagent and the amount of plasmid DNA added per well.

Action 2 : Preferably use a DNA preparation at a concentration of 0.3 to 1 µg/µl.

Action 3 : Use high-quality plasmid preparation, free of proteins and RNA (OD260/280 > 1.8).

Cellular toxicity

Action 1 : Analyze transfection at an earlier time point (e.g. at 24 h instead of 48 h).

Action 2 : Decrease the amount of plasmid DNA added per well.

Action 3 : Decrease the volume of MesGen reagent.

Action 4 : Ensure that the plasmid preparation is endotoxin-free.

3 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

4 FORMULATION AND STORAGE

MesGen™ Transfection Reagent is shipped at room temperature but should be stored at 4°C upon arrival to ensure long term stability. Reagent, as guaranteed and indicated on the Certificate of Analysis, is stable for 6 months to at least one year when stored appropriately.