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| ***MESGEN.pngMesGenTM Transfection Reagent*** |

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

***DESCRIPTION***

*MesGenTM Transfection Reagent is a novel molecule based on a polymer formulation manufactured at Polyplus-transfection. MesGenTM ensures effective and reproducible DNA transfection into mammalian cells. MesGenTM 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 106 - 2 x 106 | 75 - 78.5 | 10 |
| 150 mm / flask 175 cm2 | 2 x 106 - 5 x 106 | 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 MesGenTM 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***

*MesGenTM 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.*

