Rev. 1

ScienCell™
Research Laboratories

MesenFectagen (MesenF)

Catalog #0933

250 transfections in 96-well plate

Product Description

The delivery of foreign DNA into eukaryotic cells is one of the most common molecular biology techniques used to study biological mechanisms. However, unlike transformed cell lines, efficient transfection of primary cells can be challenging. MesenFectagen is a cationic polymer-based transfection system specifically designed and optimized for primary mesenchymal stem cells. It can be used in the presence of serum and antibiotics and features a simple protocol compatible with both serum-containing and serum-free conditions. The reagent supports multiple culture formats (6-, 12-, 24-, and 96-well plates), and an optimized one-day transfection procedure can be used instead of a conventional two-day workflow, providing time-saving and highly reproducible transfection.

Kit Components

Cat#	Component	Quantity	Storage
0933a	MesenFectagen Reagent A	1 vial	-20°C
0933b	MesenFectagen Reagent B	1 vial	-20°C

Materials Supplied by User (Not provided)

- Primary mesenchymal stem cells
- Complete growth medium suitable for the chosen cell
- DNA plasmid, siRNA.
- Cell culture plates (6-, 12-, 24-, or 96-well)
- Standard tissue culture equipment (pipettes, incubator, etc.)
- pSV-β-Galactosidase control vector
- Poly-L-lysine (PLL)
- Deionized H_2O

Product Use

MesenF is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control

MesenFectagen performance was validated using Human Mesenchymal Stem Cells (HMSCs, Cat. No. 7500, ScienCell™) transfected with Promega® pSV-β-Galactosidase control vector. Typically, ~20-35% transfection efficiency was achieved 24 hours post-transfection, as measured by X-Gal staining (Figure 1).

Shipping and Storage
Upon receipt, aliquot and store at -20°C, avoid repeated freezing/thawing cycles. Product is shipped on dry ice.

Procedures for Transfecting Adherent Cells (Example for 96-well Plate)

1. **Preparation of Coverslips and Cell Seeding**
   - **1.1.** Coat 96-well plates containing sterile cover glasses with poly-L-lysine (PLL) at a final concentration of 2 μg/cm².
   - **1.2.** Incubate at 37°C for 2–4 hours, then rinse twice with sterile deionized H_2O and allow the surface to dry inside the biosafety cabinet, before seeding of cells.
   - **1.3.** Harvest mesenchymal stem cells at ~60–80% confluency and dilute cells in Mesenchymal Stem Culture Medium to give a final concentration of ~1.1 x 10^5 cells/mL.
     Note: The pre-coating of poly-l-lysine ensures good and even cell adhesion.

2. **Preparation of DNA–MesenFectagen Complexes**
   - **2.1.** Prepare plasmid DNA stock at 1 μg/μL in sterile deionized H_2O.
   - **2.2.** For each well of a 96-well plate:
   - **2.3.** Add 0.5 μL plasmid DNA, 10.5 μL sterile deionized H_2O, and 5 μL MesenFectagen Reagent B to a sterile microtube.
   - **2.4.** Vortex gently and spin down briefly.
   - **2.5.** Add 4 μL MesenFectagen Reagent A, to make the total volume of the transfection mixture to be 20 μL.
   - **2.6.** Vortex for 5 seconds, and spin down again.
   - **2.7.** Incubate the transfection mixture for 20–30 minutes at room temperature to allow complex formation.
     Note: Complex formation in sterile deionized H_2O *provides optimal complex and reproducible efficiency. Serum-free medium can also be used.*

3. **Transfection**
   - **3.1.** Plate 180 μl of cell suspension (~1.1×10^5 cells/ml) in each well to give ~2×10^4 cells per well.
   - **3.2.** Add the transfection mixture (total 20 μL) dropwise to each well.
   - **3.3.** Gently rock the plate side-to-side to ensure even distribution of complexes over the cells.
   - **3.4.** Perform a medium change after 4-6 hours incubation with transfection mixtures, replace with 200 μL fresh culture medium.
   - **3.5.** Incubate under standard conditions (37°C, 5% CO_2) for additional 16-18 hours.

4. **Post-Transfection Analysis**
   - **4.1.** Harvest cells 24 hours post transfection and assay for gene expression.

   Note: Cell and reagent amounts listed are recommended for 96-well plates. For larger wells, scale up of mesenchymal stem cells and transfection reagents (DNA, sterile deionized H_2O, MesenFectagen A&B) proportionally according to well surface area (Table 1).

Table 1. Recommended quantities of immortalized cells and MesenFectagen reagents per well.

Culture Vessel	Growth Area (cm^2/well)	# of Cells	1 µg/µL DNA stock (µL)	Sterile DI H_2O (µl)	MesenFectagen Reagent B (µL)	MesenFectagen Reagent A (µL)	Medium (µL)
96-well plate	0.35	20,000	0.5	10.5	5	4	180
48-well plate	0.8	45,000	1.1	24	11.4	9.1	411
24-well plate	2.0	115,000	2.9	60	29	23	1029
12-well plate	4.0	230,000	5.7	120	57	46	2057
6-well plate	9.6	550,000	13.7	288	137	110	4937

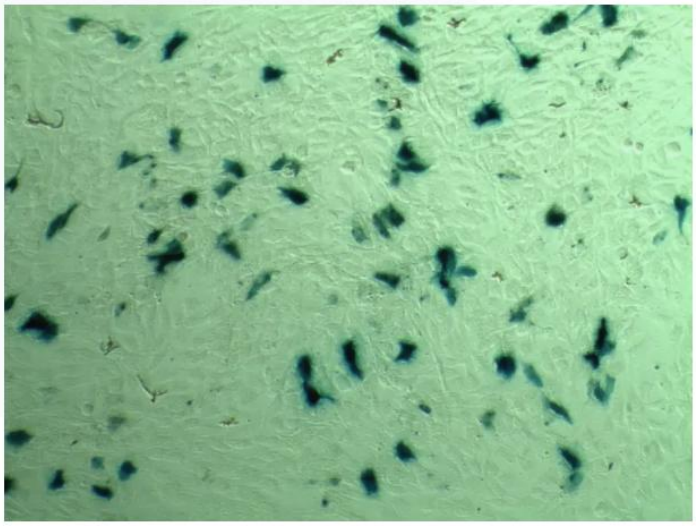

Figure 1: HMSCs expressing β-galactosidase after transfection using MesenFectagen.

Troubleshooting Table

Problem	What it means	Possible Cause	Solution
Low efficiency	Few transfected cells (<50% X-gal–positive cells)	Incorrect DNA: reagent ratio	Increase Reagent B by 20%; test ±20% of DNA amount from Table 1
High cytotoxicity	>20-30% cell death post-transfection	Reagent A overdose or no serum	Reduce Reagent A by 10%; replace medium at 4h
Aggregates/precipitates	Clumps in mix or uneven cell coverage	Excessive vertexing of reagents	Pipette gently; use fresh DI H_2O
Slow/weak expression	Low signal even at 48h	Too much media volume	Reduce well media to 400 µL before complex