

**SMCFectagen
(SMCF)**

Catalog #0953

*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. SMCFectagen is a cationic polymer-based transfection system specifically designed and optimized for primary smooth muscle 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
0953a	SMCFectagen Reagent A	1 vial	-20°C
0953b	SMCFectagen Reagent B	1 vial	-20°C

Materials Supplied by User (Not provided)

- Primary smooth muscle 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 H₂O

Product Use

SMCF 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

SMCFectagen performance was validated using Human Aortic Smooth Muscle Cells (HASMCs, Cat. No. 6110, ScienCell™) transfected with Promega® pSV- β -Galactosidase control vector. Typically, ~20-40% 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 $2\text{ }\mu\text{g}/\text{cm}^2$.
- 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 smooth muscle cells at $\sim 60\text{--}80\%$ confluency and dilute cells in Smooth Muscle Cell Culture Medium to give a final concentration of $\sim 8.0 \times 10^4$ cells/mL.

Note: The pre-coating of poly-l-lysine ensures good and even smooth muscle cells adhesion.

2. Preparation of DNA–SMCFectagen Complexes

- 2.1. Prepare plasmid DNA stock at $1\text{ }\mu\text{g}/\mu\text{L}$ in sterile deionized H_2O .
- 2.2. For each well of a 96-well plate:
- 2.3. Add $0.5\text{ }\mu\text{L}$ plasmid DNA, $12.5\text{ }\mu\text{L}$ sterile deionized H_2O , and $2\text{ }\mu\text{L}$ SMCFectagen Reagent B to a sterile microtube.
- 2.4. Vortex gently and spin down briefly.
- 2.5. Add $5\text{ }\mu\text{L}$ SMCFectagen Reagent A, to make the total volume of the transfection mixture to be $20\text{ }\mu\text{L}$.
- 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 $180\text{ }\mu\text{L}$ of cell suspension ($\sim 8.0 \times 10^4$ cells/mL) in each well to give $\sim 1.5 \times 10^4$ cells per well.
- 3.2. Add the transfection mixture (total $20\text{ }\mu\text{L}$) dropwise to each well.
- 3.3. Gently rock the plate side-to-side to ensure even distribution of complexes over the cells.
- 3.4. Incubate under standard conditions (37°C , $5\%\text{ CO}_2$) for 24 hours.

Optional: Medium can be replaced after 4–6 hours to minimize cytotoxicity. Longer incubation increases efficiency but reduces viability.

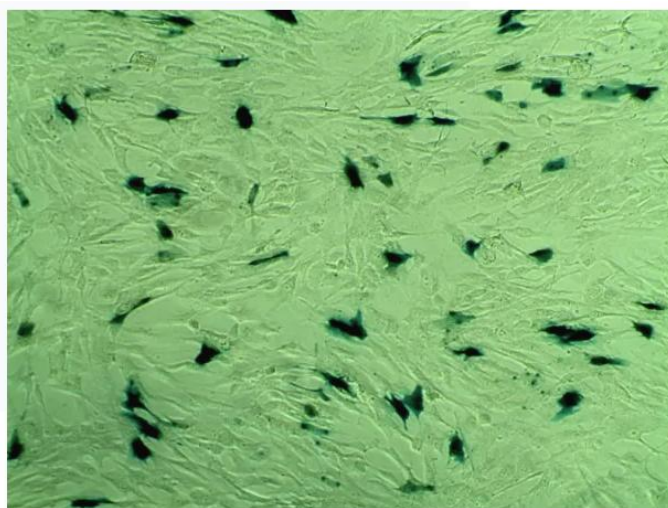
4. Post-Transfection Analysis

- 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 smooth muscle cells and transfection reagents (DNA, sterile deionized H_2O , SMCFectagen A&B) proportionally according to well surface area (Table 1).

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

Culture Vessel	Growth Area (cm ² /well)	# of Cells	1 µg/µL DNA stock (µL)	Sterile DI H ₂ O (µl)	SMCfectagen Reagent B (µL)	SMCfectagen Reagent A (µL)	Medium (µL)
96-well plate	0.35	20,000	0.5	12.5	2	5	180
48-well plate	0.8	45,000	1.1	29	4.6	11.4	411
24-well plate	2.0	115,000	2.9	71	11.4	29	1029
12-well plate	4.0	230,000	5.7	143	23	57	2057
6-well plate	9.6	550,000	13.7	343	55	137	4937

**Figure 1:** HASMCs expressing β -galactosidase after transfection using SMCfectagen.**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 $\pm$ 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 vortexing of reagents	Pipette gently; use fresh DI H ₂ O
Slow/weak expression	Low signal even at 48h	Too much media volume	Reduce well media to 400 µL before complex