Rev. 1

ScienCell™ Research Laboratories

UniFectagen (UniF)

Catalog #0983

100 transfections in 96-well plate

Product Description

UniFectagen is a high-efficiency transfection reagent optimized for a wide range of immortalized mammalian cell lines, including HEK293T and CHO. It enables rapid and reproducible delivery of plasmid DNA, siRNA, and lentiviral vector systems into both adherent and suspension cells, achieving high transfection efficiency with minimal cytotoxicity. The reagent features a simple protocol compatible with both serum and serum-free conditions and supports multiple culture formats (6-, 12-, 24-, and 96-well plates) for applications such as reporter assays, gene expression studies, lentivirus packaging, and functional screening.

Kit Components

Cat#	Component	Quantity	Storage
0983a	UniFectagen Reagent A	1 vial	-20°C
0983b	UniFectagen Reagent B	1 vial	-20°C

Materials Supplied by User (Not provided)

- Immortalized cells (e.g., HEK293, CHO, HeLa, etc.)
- Complete growth medium suitable for the chosen cell line
- DNA plasmid, siRNA, or lentiviral packaging plasmids
- Cell culture plates (6-, 12-, 24-, or 96-well)
- Standard tissue culture equipment (pipettes, incubator, etc.)
- Poly-L-lysine (PLL)
- GFP plasmid
- Deionized H_2O

Product Use

UniF 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

UniFectagen performance was validated using HEK293T cells transfected with a plasmid encoding green fluorescent protein (GFP). Cells exhibited strong fluorescence positivity and viability 24 hours post-transfection. Figure 1: A. Non-transfected control cells showing minimal background fluorescence. B. Cells transfected with GFP plasmid using UniFectagen, exhibiting strong and uniform GFP expression.

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

Procedure (Example for 24-well Plate)

1. **Preparation of Coverslips and Cell Seeding**
   - **1.1.** Coat 24-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.
   - **1.3.** Harvest immortalized cells (e.g., HEK293T or CHO) at ~70–80% confluency and resuspend in complete growth medium.
   - **1.4.** Seed 80,000–100,000 cells per well in a final volume of 1 mL complete medium.
   - **1.5.** Incubate overnight to allow proper cell attachment and spreading on the PLL-coated cover glass.

2. **Preparation of DNA–UniFectagen Complexes**
   - **2.1.** Prepare plasmid DNA stock at 1 μg/μL in sterile deionized H_2O.
   - **2.2.** For each well of a 24-well plate:
   - **2.3.** Add 0.5 μL plasmid DNA, 15 μL sterile deionized H_2O, and 8 μL UniFectagen Reagent B to a sterile microtube.
   - **2.4.** Vortex gently and spin down briefly.
   - **2.5.** Add 20 μL UniFectagen Reagent A, vortex for 5 seconds, and spin down again.
   - **2.6.** Incubate the transfection mixture for 15–20 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.** Add the transfection mixture (total 43.5 μL) dropwise to each well containing 600 μL of complete medium.
   - **3.2.** Gently rock the plate side-to-side to ensure even distribution of complexes over the cells.
   - **3.3.** Incubate under standard conditions (37°C, 5% CO_2) for 24–48 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.** For plasmid DNA encoding GFP, observe cells under a fluorescence microscope 24 hours post-transfection to assess expression.
   - **4.2.** Cells on cover glass can be directly used for imaging, immunofluorescence, or live-cell analysis.
     Note: Cell and reagent amounts listed are recommended for 24-well plates. For larger wells, scale up cells and transfection reagents (DNA, sterile deionized H_2O*, UniFectagen A&B) proportionally according to well surface area (Table 1).*

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

Culture Vessel	Growth Area (cm^2/well)	# of Cells	1 µg/µL DNA stock (µL)	Sterile DI H_2O (µl)	UniFectagen Reagent B (µL)	UniFectagen Reagent A (µL)	Medium (µL)
96-well plate	0.35	15,000–20,000	0.1	4	0.5	2	100
48-well plate	0.8	35,000–40,000	0.23	9.1	1.1	4.5	220
24-well plate	2.0	80,000–100,000	0.5	15	8	20	600
12-well plate	4.0	160,000–200,000	1.0	30	15	40	1200
6-well plate	9.6	400,000–500,000	2.4	72	36	95	2800

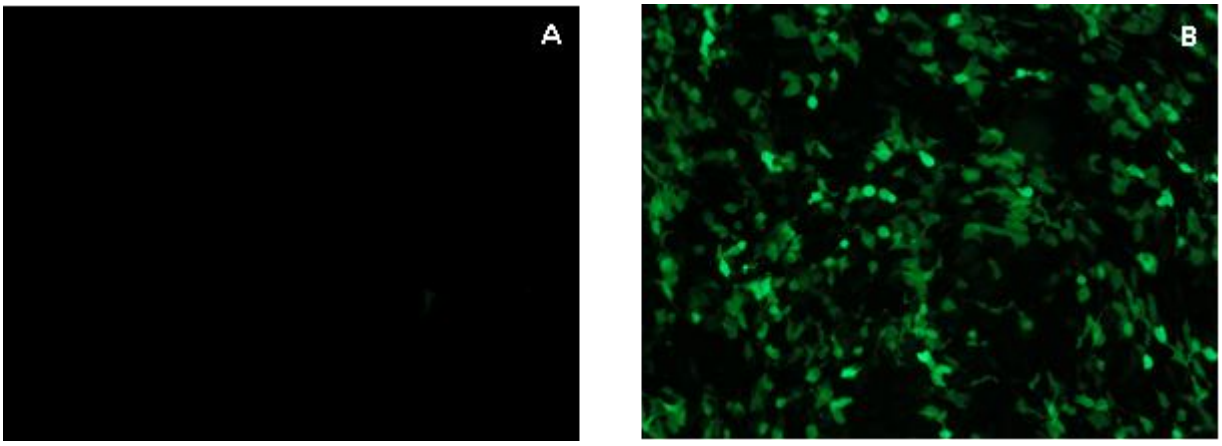

Figure 1: HEK293T cells (24-well, cover glass). **A.** Non-transfected control (minimal fluorescence). **B.** GFP-transfected with UniFectagen (strong, uniform expression at 24h).

Troubleshooting Table

Problem	What it means	Possible Cause	Solution
Low efficiency	Few transfected cells (<50% GFP+)	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