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Cellfectin® Reagent

Cat. No. 10362-010 **Size: 1 ml**
Store at +4°C (do not freeze)

Description

Cellfectin® Reagent is suitable for the transfection of DNA into insect and mammalian cells, and is a 1:1.5 (M/M) liposome formulation of the cationic lipid N, N^{I}, N^{II}, N^{III}-Tetramethyl- N, N^{I}, N^{II}, N^{III}-tetrapalmityl-spermine (TM-TPS), and dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water. Cellfectin® has been found to be superior for the transfection of Sf9 cells. Refer to the Cell Lines database at www.invitrogen.com for a list of other cell types successfully transfected.

Important Guidelines for Transfection

1. Form complexes using the amounts of DNA and Cellfectin® recommended (see pages 2-3). Optimize as necessary. **Note:** We recommend diluting DNA and Cellfectin® into Sf-900 II SFM (for insect cells; Cat. No. 10902-096) or Opti-MEM® I Reduced Serum Medium (for mammalian cells; Cat. No. 31985-062) before complexing.
2. Cellfectin® is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5-10 times before use.
3. Transfect cells at the confluence or cell density recommended (see pages 2-3). Optimize as necessary. Maintain the same seeding conditions between experiments.
4. **Do not** add antibiotics to media during transfection as this causes cell death.
5. For optimal results, perform transfection in medium without serum. Cells may be transfected in the presence of serum, if desired; however, complexes **must** be formed in serum-free medium.
6. Test serum-free media for compatibility with Cellfectin® since some serum-free formulations may inhibit cationic lipid-mediated transfection.
7. Procedures are provided to transfect cells in a 6-well format (60-mm format for stable mammalian cell transfection). For other formats, vary the amounts of DNA, Cellfectin®, cells, and medium used in proportion to the relative surface area of the tissue culture vessel.

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Transfecting Insect Cells with Baculovirus DNA

Use this procedure to transfect Sf9 insect cells in a **6-well format**. All amounts and volumes are given on a per well basis.

1. Plate 9 x 10^5 Sf9 cells in 2 ml of Sf-900 II SFM containing antibiotics (*i.e.* penicillin/streptomycin/neomycin; Cat. No. 15640-055) at 0.5X final concentration. Allow cells to attach for at least 1 hour.
2. **For each transfection sample**, prepare complexes as follows:
   a. Dilute 1-2 µg of baculovirus DNA in 100 µl of Sf-900 II SFM without antibiotics.
   b. Mix Cellfectin® before use, then dilute 1.5-9 µl in 100 µl of Sf-900 II SFM without antibiotics.
   c. Combine the diluted DNA with diluted Cellfectin® (total volume = 200 µl). Mix gently and incubate for 15-45 minutes at room temperature (solution may appear cloudy).
3. Remove the growth medium from the cells and wash once with Sf-900 II SFM without antibiotics. Remove the wash medium.
4. Add 0.8 ml of Sf-900 II SFM to the complexes (Step 2c), mix gently and add to the cells. Incubate cells at 27°C for 5 hours.
5. Remove the transfection mixture and replace with 2 ml of Sf-900 II SFM containing antibiotics. Incubate cells at 27°C for 48 hours.
6. Assay for gene activity at 48 hours post-transfection; harvest virus at 72 hours.

Transfecting Adherent Mammalian Cells

Use the following procedure to transiently or stably transfect mammalian cells. All amounts and volumes are given on a per well basis.

1. One day before transfection, plate cells in growth medium without antibiotics such that they will be at the recommended confluence at the time of transfection.

Condition	Cell no.	Growth med. vol.	Format	Confluence at txfn
Transient	1-2 x 10^5	2 ml	6-well	60-80%
Stable	1-2 x 10^5	4 ml	60-mm	30-50%

2. **For each transfection sample**, prepare complexes as follows:
   a. Dilute 1-2 µg of DNA in 100 µl of Opti-MEM® I Reduced Serum Medium (or other medium) without serum.
   b. Mix Cellfectin® before use, then dilute 2-15 µl of Cellfectin® in 100 µl of Opti-MEM® I Medium (or other medium) without serum.
   c. Combine the diluted DNA with diluted Cellfectin® (total volume = 200 µl). Mix gently and incubate for 10-15 minutes (15-45 minutes for stable transfection) at room temperature (solution may appear cloudy).
3. Remove the growth medium from the cells and wash once with 2 ml of growth medium without serum. Remove the wash medium.
4. Add 0.8 ml of Opti-MEM® I Medium without serum (1.8 ml for stable transfection) to the complexes (Step 2c), mix gently and add to cells.
5. Incubate cells at 37°C in a CO_2 incubator for 5-24 hours.
6. Replace the medium with 2 ml of growth medium containing serum (4 ml for stable transfection).
7. **Transient:** Test for transgene expression 24-72 hours post-transfection. **Stale cell lines:** Passage cells at a 1:5 (or higher dilution) into selective medium 48-72 hours post-transfection.

Transfecting Suspension Mammalian Cells (6-well format)

1. For each transfection sample, form complexes as in Step 2 of the protocol above, with the following exceptions: (1) Dilute DNA and Cellfectin® into 0.5 ml of Opti-MEM® I Medium each and (2) Incubate complexes for 15-45 minutes at room temperature.
2. For each transfection sample, centrifuge 2 x 10^6 cells and aspirate medium.
3. Resuspend the cell pellet with the DNA-lipid complex solution (Step 1) and transfer to a well of a 6-well plate. Incubate at 37°C in a CO_2 incubator for 4-5 hours.
4. Add 0.5 ml of growth medium containing 30% fetal bovine serum. **Note:** Add PMA and/or PHA, if desired, to enhance promoter activity and gene expression.
5. The following day, add 1-2 ml of complete growth medium. Assay for transgene expression 24-48 hours post-transfection.

Optimizing Transfections

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying cell density, DNA and Cellfectin® concentrations, and transfection incubation time.

Quality Control

Cellfectin® is tested for the absence of microbial contamination using blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection of CHO-K1 cells with a reporter plasmid.

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