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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', N''-Tetramethyl- N, N', N''-tetrapalmityl-spermine (TM-TPS), and dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water. Cellfectin® has been found to be superior for the transfection of S9 cells. Refer to the Cell Lines database at www.invitrogen.com for a list of other cell types successfully transfected.

Important Guidelines for Transfection

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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.

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Part No.: 10362.pps

Rev. Date: 16 July 2004

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For technical questions about this product, call the Invitrogen Tech-Line® U.S.A. 800 955 6288

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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 2 ml of growth medium without antibiotics. Remove the wash medium.

4. Add 0.5 ml of Opti-MEM® I Reduced Serum Medium (or other medium) without serum.

5. The following day, add 1-2 ml of complete growth medium without antibiotics. Incubate cells at 27°C for 5 hours.

6. Replace the medium with 2 ml of growth medium containing 30% fetal bovine serum.

7. Test for transgene expression 24-72 hours post-transfection.

Stable 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 10^5 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 CO2 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.

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.

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) to 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 CO2 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.

Stable cell lines: Passage cells at a 1:5 (or higher dilution) into selective medium 48-72 hours post-transfection.

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.

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) to 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.

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5. Incubate cells at 37°C in a CO2 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.

Stable 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 10^5 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 CO2 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.
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 2 ml of SF-900 II SFM containing antibiotics.
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.
6. Assay for gene activity at 48 hours post-transfection; harvest virus at 72 hours.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell no.</th>
<th>Growth med. vol.</th>
<th>Format</th>
<th>Confluence at txfn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient</td>
<td>1-2 x 10^5</td>
<td>2 ml</td>
<td>6-well</td>
<td>60-80%</td>
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<tr>
<td>Stable</td>
<td>1-2 x 10^5</td>
<td>4 ml</td>
<td>60-mm</td>
<td>30-50%</td>
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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.
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.
4. Replace the medium with 2 ml of growth medium containing serum (4 ml for stable transfection).
5. Incubate at 37°C in a CO2 incubator for 4-5 hours.
6. Remove 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. Stable 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 CO2 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.

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