invitrogen
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Optifect™ Reagent

Part no. 12579017.pps MAN0001097 **Rev. Date** 13 June 2011

Cat. no. 12579-017 **Size** 1 mL **Store at 4°C (do not freeze)**

Description

Optifect™ Reagent is a proprietary formulation for transfecting nucleic acids into eukaryotic cells, and is designed for optimal transfection of cells plated at low densities. Using Optifect™ Reagent for transfection provides the following advantages:

- High transfection efficiency in many cell types and cell lines. Refer to the Cell Lines database at **www.lifetech.com** for a list of cell types transfected.
- Ideally suited for applications where long duration of transgene expression is preferred (e.g. proliferation assays or cell cycle studies).
- DNA-Optifect™ Reagent complexes can be added directly to cells in culture medium.
- It is not necessary to remove complexes or change/add medium after transfection, but complexes may be removed after 4–6 hours.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website. Go to **www.lifetech.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Important Guidelines for Transfection

- **Transfect cells at 30–70% confluence.** Optimization may be necessary. Maintain the same seeding conditions between experiments.
- We recommend using Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) to dilute DNA and Optifect™ Reagent before complexing.
- **Do not** add antibiotics to media during transfection as this causes cell death.
- Test serum-free media for compatibility with Optifect™ Reagent since some serum-free formulations (e.g. CD 293, 293 SFM II, CD Hybridoma) may inhibit cationic lipid-mediated transfection.

Prepare Cells for the Transfection Procedure

Use the following procedure to transfect mammalian cells in a **24-well format**. For other formats, see **Scaling Up or Down Transfections**. All amounts and volumes are given on a per well basis.

- **Adherent cells:** One day before transfection, plate cells in 500 µL of growth medium without antibiotics so that they will be 30–70% confluent at the time of transfection.
- **Suspension cells:** Just prior to preparing complexes, plate $2–4 \times 10^5$ cells in 500 µL of growth medium without antibiotics.

Transfection Procedure

1. **For each transfection sample**, prepare complexes as follows:
   a. Dilute 0.8–1.2 µg of DNA in 50 µL of Opti-MEM® I Reduced Serum Medium without serum. Mix gently.
   b. Mix Optifect™ Reagent gently before use, then dilute 1–4 µL in 50 µL of Opti-MEM® I Medium. Mix gently and incubate for 5 minutes at room temperature.

      Note: Combine diluted Optifect™ Reagent with diluted DNA within 30 minutes.
   c. After 5 minute incubation, combine the diluted DNA with diluted Optifect™ Reagent (total volume = 100 µL). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

      Note: Complexes are stable for 6 hours at room temperature.
2. Add the 100 µL of complexes to a well containing cells and medium. Mix gently by rocking the plate back and forth.
3. Incubate cells at 37°C in a CO_2 incubator for 24–72 hours prior to testing for transgene expression. It is not necessary to change the medium, but medium may be replaced after 4–6 hours.

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying cell density and the amounts of DNA and Optifect™ Reagent used. For cell lines that are particularly sensitive to transfection-mediated cytotoxicity (e.g. HeLa, HT1080), use the lower amounts of Optifect™ Reagent suggested in the table on page 4 (see column 5).

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Optifect™ Reagent, DNA, cells, and medium used in proportion to the relative surface area, as shown in the following table. With automated, high-throughput systems, a complexing volume of 50 µL is recommended for transfections in 96-well plates.

Culture vessel	Relative surf. area vs. 24-well	Vol. of plating medium	DNA (µg) in media vol. (µL)	Optifect™ Reagent (µL) in media vol. (µL)
96-well	0.2	100 µL	0.25–0.35 µg in 25 µL	0.5–2.0 µL in 25 µL
48-well	0.4	300 µL	0.6–1.0 µg in 50 µL	0.5–3.0 µL in 50 µL
24-well	1	500 µL	0.8–1.2 µg in 50 µL	1.0–4.0 µL in 50 µL
12-well	2	1 mL	3.0–4.0 µg in 100 µL	7–14 µL in 100 µL
6-well	5	2 mL	3.5–4.5 µg in 250 µL	12–24 µL in 250 µL
10-cm	30	15 mL	21–27 µg in 1.5 mL	50–70 µL in 1.5 mL

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