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# *PrimeFect™ Transfection Reagent*

Cat. #: M0003 Size: 1 ml/3 ml

## Introduction:

***PrimeFect™ Transfection Reagent*** is a lipid-based transfection reagent that forms a complex with DNA or RNA, and transports the complex into a variety of adherent and suspension cell lines. PrimeFect™ Transfection Reagent has been tested to work the same efficiency as Lipofectamine® 2000 Reagent, and used for the transfection of both DNA and RNA into eukaryotic cells even in the presence of serum.

## Key Features:

1. Superior transfection efficiency for a broad range of cell lines.
2. Does not require removal of serum or culture medium.
3. Does not require washing or changing of medium after transfection.
4. Low cytotoxicity.

## Protocols

### DNA Transfection

Use the following procedure to transfect DNA into 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. Prepare complexes using a DNA (µg) to PrimeFect™ (µl) ratio of 1:2 to 1:3 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see Optimizing DNA Transfection).

1. **Adherent cells**: One day before transfection, plate 0.5-2×$10^5$ cells in 500 µl of growth medium without antibiotics so that cells will be 70-90% confluent at the time of transfection.

**Suspension cells**: Just prior to preparing complexes, plate 4-8×$10^5$ cells in 500 µl of growth medium without antibiotics.

2. For each transfection sample, prepare complexes as follows:

a. Dilute DNA in 50 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.

b. Mix PrimeFect™ gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature.

**Note**: Proceed to Step c within 25 minutes.

c. After the 5 minute incubation, combine the diluted DNA with diluted PrimeFect™ (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.

3. Add the 100 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

4. Incubate cells at 37°C in a $CO_2$ incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.

5. **For stable cell lines**: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.

### Optimizing DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and PrimeFect™ concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): PrimeFect™ (µl) ratios from 1:0.5 to 1:5.

**Table 1. A Guideline for Optimal DNA Per Well in Different Culture Formats**

| Culture Dishes | Surface Area ($cm^2$) | Plating medium volume | Dilution medium volume | DNA | PrimeFect™ |
|---|---|---|---|---|---|
| 96-well | 0.3 | 100 µl | 2× 25 µl | 0.2 µg | 0.5 µl |
| 24-well | 2 | 500 µl | 2× 50 µl | 0.8 µg | 2.0 µl |
| 12-well | 4 | 1 ml | 2× 100 µl | 1.6 µg | 4.0 µl |
| 6-well | 10 | 2 ml | 2× 250 µl | 4.0 µg | 10 µl |
| 6-cm dish | 20 | 5 ml | 2× 500 µl | 8.0 µg | 20 µl |
| 10-cm dish | 60 | 15 ml | 2× 1.5 ml | 24 µg | 60 µl |

### RNA Transfection

Use the following procedure to transfect RNA into 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. Use this procedure as a starting point; optimize transfections as described in Optimizing RNA Transfection.

1. One day before transfection, plate cells in 500 µl of growth medium without antibiotics such that they will be 30-50% confluent at the time of transfection.

**Note**: Transfecting cells at a lower density allows a longer interval between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth.

2. For each transfection sample, prepare oligomer- PrimeFect™ complexes as follows:

a. Dilute 20 pmol Stealth™ RNAi or siRNA oligomer in 50 µl Opti-MEM® I Reduced Serum Medium without serum (final concentration of RNA when added to the cells is 33 nM). Mix gently.

**Storage:** PrimeFect™ In Vitro DNA Transfection Reagent is stable for up to 12 months at 4°C.

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b. Mix PrimeFect™ gently before use, then dilute 1 µl in 50 µl Opti- MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature.

**Note**: Proceed to Step c within 25 minutes.

c. After the 5-minute incubation, combine the diluted oligomer with the diluted PrimeFect™. Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

3. Add the oligomer- PrimeFect™ complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth. Incubate the cells at 37°C in a $CO_2$ incubator for 24-96 hours until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

## Optimizing RNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and PrimeFect™ concentrations. Test 10-50 pmol RNA and 0.5-1.5 µl PrimeFect™ for 24-well format. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions.

## Scaling Up or Down Transfections

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

**Note**: You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of complexes.

**Table 2. A Guideline for Optimal RNA Per Well in Different Culture Formats**

| Culture Dishes | Surface Area ($cm^2$) | Plating medium volume | Dilution medium volume | RNA | PrimeFect™ |
|---|---|---|---|---|---|
| 96-well | 0.3 | 100 µl | 2× 25 µl | 5 pmol | 0.25 µl |
| 24-well | 2 | 500 µl | 2× 50 µl | 20 pmol | 1.0 µl |
| 12-well | 4 | 1 ml | 2× 100 µl | 40 pmol | 2.0 µl |
| 6-well | 10 | 2 ml | 2× 250 µl | 100 pmol | 5 µl |
| 6-cm dish | 20 | 5 ml | 2× 500 µl | 200 pmol | 10 µl |
| 10-cm dish | 60 | 15 ml | 2× 1.5 ml | 600 pmol | 30 µl |