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PolyFect™ Transfection Reagent

Catalog Number: FP330, FP331

Table 1. Product Package and Storage

Cat No.	Product Name	Amount	Storage
FP330	PolyFect™	1 mL	4°C: one year
FP331	PolyFect™	3 mL	

Introduction

PolyFect™ Transfection Reagent is a biodegradable polymer based transfection reagent that forms a complex with DNA, and transports the complex into a variety of adherent and suspension cell lines. A remarkable feature of the reagent is the rapid and complete degradation of polymer after transfection complex endocytosis, leading to much less cytotoxicity.

Feature

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

Protocols

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 PolyFect™ (µl) ratio of 1:2 to 1:3 for most cell lines. Optimization may be necessary (see Optimizing Transfection).

1. **Adherent cells:** One day before transfection, plate 0.5-2 x 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 x 10^5 cells in 500 µl of growth medium without antibiotics.
2. For each transfection sample, prepare complexes as follows:

   a. Dilute 0.8 µg DNA in 50 µl of serum-free DMEM (or other medium without serum). Mix gently.

   b. Mix PolyFect™ gently before use, then dilute 2 µl of PolyFect™ in 50 µl of serum-free DMEM.

   c. Add the diluted PolyFect™ reagent to the diluted DNA solution all at once. Mix gently and incubate for 10~15 minutes 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 Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and PolyFect™ concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): PolyFect™ (µl) ratios from 1:1 to 1:4.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of PolyFect™, DNA, 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.

Culture vessel	Surface area (cm^2)	Plating medium volume	Dilution medium volume	DNA	PolyFect™
96-well	0.3	100 µl	2 × 10 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 × 150 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