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# PolyFectVIR™ Transfection Reagent

## Catalog Number: FP317-1, FP317-2, FP317-3

**Table 1. Product Package and Storage**

| Cat No. | Product Name | Amount | Storage |
|---|---|---|---|
| FP317-1 | PolyFectVIR™ | 1 mL | 4°C: one year |
| FP317-2 | PolyFectVIR™ | 10 mL | |
| FP317-3 | PolyFectVIR™ | 100 mL | |

## Introduction

PolyFectVIR™ Transfection Reagent is an innovative cationic nanotechnology developed for the gene and cell therapy field to improve virus production. This ready-to-use chemically defined reagent is guaranteed free of components of animal origin. PolyFectVIR™ Transfection Reagent is dedicated for medium to large scale bioproduction of virus through an optimized structure and improved transfection parameters.

### Feature

- Chemically well defined.
- Co-transfection of multiple plasmids.
- High viral titer.
- Low cytotoxicity.

## Protocols

### 1. Transfection protocol for virus production in suspension cells

PolyFectVIR™ Transfection Reagent is suited for DNA transfection of cells grown in suspension in shaker flasks, spinners, cell culture bags or bioreactors in serum-free media. PolyFectVIR™ Transfection Reagent is compatible with the use of antibiotics in the cell culture medium.

#### 1.1 Cell seeding

On the day of cell seeding, adjust cell density according to your process to reach the exponential growth phase with a viable cell density (VCD) around 2- 2.5 × $10^6$ cells/mL at the time of transfection.

Cell seeding has to be adjusted according to the culture vessel you are using:

- Shake flask: we recommend seeding 1 × $10^6$ cells/mL to ensure that cells reach the optimal VCD after 24 hours;
- Other (e.g. culture bags, or bioreactors): the day of transfection will depend on the growth time needed for your cells to reach a VCD of 2- 2.5 × $10^6$ cells/mL.

#### 1.2 Preparation of the complexes

The following protocol is given for the transfection of plasmids into suspension cells for virus production. For co-transfection of multiple plasmids, the suitable plasmid ratio depends on the virus produced, the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid.

The recommended complexation parameters are described in Table 1.

**Table 1. Complexation parameters for the transfection of suspension cells.**

| Parameter | Recommended condition | Range of optimization |
|---|---|---|
| DNA amount (per $10^6$ cells) | 1 µg DNA | 0.5 µg – 2 µg |
| Ratio (µg DNA : µL PolyFectVIR™) | 1:1 | 1:1 – 1:3 |
| Complexation time | 15 min | 10 min – 20 min |

### 1.3 Transfection

Transfection parameters (i.e. DNA amount and PolyFectVIR™ volume) have to be adjusted according to the cell density reached at the time of transfection.

The following protocol is given for transfection of HEK-293 cells in 100 mL of cell culture medium according to the recommended conditions in Table1.

1. On the day of transfection, measure the cell density and determine transfection parameters (DNA amount and PolyFectVIR™ volume per million cells) according to Table 1. For this example, we assume a VCD of $2 \times 10^6$ cells/mL.
2. Dilute 200 µg of DNA in 5 mL of Opti-MEM® I Medium without serum (or other medium without serum). Mix gently.
3. Mix PolyFectVIR™ gently before use, then dilute 200 µL of PolyFectVIR™ in 5 mL of Opti-MEM® I Medium. Mix gently.
4. Add the 5 mL PolyFectVIR™ solution onto the 5 mL DNA solution all at once. Mix immediately the solution, either by briefly vortexing it or inverting the tube few times.
5. Incubate the complexes at room temperature for 15 minutes.
6. Add the 10 mL PolyFectVIR™/DNA mix to the cells. Mix gently by rocking the flask back and forth.
7. Incubate cells at appropriate temperature, shaking and $CO_2$ levels (e.g. 37°C, 125 rpm, 8%) and harvest virus when required.

## 2. Transfection protocol for virus production in adherent cells

PolyFectVIR™ Transfection Reagent is suited for virus production in adherent cells grown in plates, flasks, roller-bottles, cell-factories and bioreactors. PolyFectVIR™ Transfection Reagent is compatible with the use of serum and antibiotics in the cell culture medium.

For co-transfection of multiple plasmids, the suitable plasmid ratio depends on the virus produced, the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid.

### 2.1 Cell seeding

Cell seeding has to be adjusted according to your cell culture vessel and process. For optimal transfection conditions with PolyFectVIR™, we recommend seeding the cells the day before transfection to reach 50-80% confluent cells on the day of transfection.

**Note:** The cell density is crucial to determine transfection condition. Thus, we recommend dedicating a separate wells or plate to measure cell density at the time of transfection.

**Table 2. Recommended number of cells to seed the day before transfection.**

| Culture vessel | Number of adherent cells to seed the day before transfection |
|---|---|
| 10 cm/T-75/T-175/multilayer cell factories | 50,000 ± 25,000 cells/$cm^2$ |

The optimal seeding is dependent on cell subclone (doubling time), culture medium and time of harvest of the virus of interest.

## 2.2 Preparation of the complexes

We recommend measuring the VCD at the time of transfection to determine optimized transfection conditions for each transfection. Calculating the amount of DNA per million of cells will improve reproducibility and the scale up of your process.

The recommended complexation parameters are described in Table 3.

**Table 3. Complexation parameters for the transfection of adherent cells.**

| Parameter | Recommended condition | Range of optimization |
|---|---|---|
| DNA amount (per $10^6$ cells) | 1.5 µg DNA | 1 µg – 2 µg |
| Ratio (µg DNA : µL PolyFectVIR™) | 1:1 | 1:1 – 1:3 |
| Complexation time | 15 min | 10 min – 20 min |

## 2.3 Transfection

The following protocol is given for transfection of HEK-293 cells in a flask 75 $cm^2$ according to the recommended conditions in Table 3.

1. On the day of transfection, measure the cell density and determine transfection parameters (DNA amount and PolyFectVIR™ volume per million cells) according to Table 3. For this example, we assume a VCD of 75,000 cells/$cm^2$.
2. Dilute 8.5 µg of DNA in 500 µL of Opti-MEM® I Medium without serum (or other medium without serum). Mix gently.
3. Mix PolyFectVIR™ gently before use, then dilute 8.5 µL of PolyFectVIR™ in 500 µL of Opti-MEM® I Medium. Mix gently.
4. Add the 500 µL PolyFectVIR™ solution onto the 500 µL DNA solution all at once. Mix immediately the solution, either by briefly vortexing it or inverting the tube few times.
5. Incubate the complexes at room temperature for 15 minutes.
6. Add the 1 mL PolyFectVIR™/DNA mix to the cells in 10 mL of medium. Mix gently by rocking the flask back and forth.
7. Incubate cells at appropriate temperature and $CO_2$ levels (e.g. 37°C, 5%) and harvest virus when required.