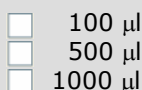


# BAPoly® In Vitro DNA Transfection Reagent

---An Advanced Protocol for Transfecting Hard-to-Transfect Cells



13 Taft Ct, Rockville, MD, 20850  
TEL: 1-(734)-604-2386  
Email: support@BioArkTech.com  
Web: www.BioArkTech.com

This product is for laboratory research ONLY and not for diagnostic use

## Introduction:

Based on our innovative polymer synthesis technology, BAPoly® DNA In Vitro Transfection Reagent is formulated to be a powerful transfection Reagent that ensures effective and reproducible transfection with less cytotoxicity. BAPoly® reagent was shown to deliver genes to various established cell lines as well as primary cells.

## Procedures for Transfecting Hard-to-Transfect Cells:

### Step I. Culturing of Cells Before Transfection

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

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

Culture Dishes	Surface Area (cm <sup>2</sup> )	Optimal Cell Number
T75 Flask	75	9.6 × 10 <sup>6</sup>
100 mm Dish	58	7.3 × 10 <sup>6</sup>
60 mm Dish	21	2.7 × 10 <sup>6</sup>
35 mm Dish	9.6	1.0 × 10 <sup>6</sup>
6-well Plate	9.6	1.0 × 10 <sup>6</sup>
12-well Plate	3.5	0.44 × 10 <sup>6</sup>
24-well Plate	1.9	0.24 × 10 <sup>6</sup>
48-well Plate	1.0	0.11 × 10 <sup>6</sup>
96-well Plate	0.3	0.31 × 10 <sup>5</sup>

**Table 2. Recommended Amounts for Different Culture Vessel Formats**

Culture Dish	Transfection Complex Volume (ml)	Plasmid DNA (µg)	BAPoly® Reagent (µL)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1	4
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	8	32
T75 flask	1.5	36	144
250 ml flask	2.5	100	400

### Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect

cells in 6-well plates, refer to **Table 1** for optimal cell number per well per culture vessels' surface area. The optimal transfection conditions are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with a complete culture medium.

**Note:** Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment

- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.

- Centrifuge the required ~1.0x10<sup>6</sup> cells per well for 6-well plate at 150xg at room temperature for 10 min.

- Use a fine tip pipette to remove the supernatant **completely** so that no residual medium covers the cell pellet.

### Step III. Preparation and application of Transfection Complex

**For most of mammalian cells, the optimal ratio of BAPoly® (µL):DNA (µg) is 4:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and BAPoly® Reagent.**

The following protocol is given for transfection in 6-well plates, refer to **Table 2** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.

- For each well of 6-well plate, dilute 8 µl of BAPoly® reagent (Ver. II) into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

- Add the diluted BAPoly® Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**

- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10~15 minutes at room temperature to allow transfection complexes to form.

**Note:** Never keep the transfection complexes longer than 20 minutes

- **Gently** resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.

- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate. Incubate at 37 °C with 5% CO<sub>2</sub>.

- Remove transfection complex containing medium **gently** and refill with complete culture medium 8~12 hours after plating.

- Check transfection efficiency 24 to 48 hours post transfection.