Cat # BAL100688 Store at 4

BAPoly® In Vitro DNA Transfection Reagent

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

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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^2)	Optimal Cell Number
T75 Flask	75	9.6×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.0×10^6
6-well Plate	9.6	1.0×10^6
12-well Plate	3.5	0.44×10^6
24-well Plate	1.9	0.24×10^6
48-well Plate	1.0	0.11×10^6
96-well Plate	0.3	0.31×10^5

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 ~1.0×10^6 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 mammalianells, 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_2.
- 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.