General Cell Transfection Protocol for BAPtofect™-25

For Plasmid DNA and Messenger RNA

Kit Contents

Item #B25002-S Size: 250 µL (**Yellow cap**)
Item #B25005-S Size: 500 µL (**Blue cap**)
Item #B25010-S Size: 1 mL (**Violet cap**)
Item #B25050-S Size: 5 x 1 mL (5 x 1 mL vials – **Violet cap**)
Item #B25100-S Size: 10 X 1 mL (10 x 1 mL vials – **Violet cap**)

BAPtofect™-25 is provided at 1 µg /µL
BAPtofect™-25 is stable at room temperature.
Recommended Storage Temperature, 4 – 25 °C. Do not freeze.

BAPtofect™-25 Transfection Protocol:

1. Seed cells so that they are 60-80% confluent at the time of transfection*.
2. Remove culture media and wash cells with Dulbecco's phosphate-buffered saline.
3. Add Opti-MEM media and place in CO_2 incubator for a few minutes prior to transfection.
4. Preparation of transfection complex:
    a) Dilute BAPtofect™-25 with Opti-MEM media.
    b) In a separate tube mix Nucleic Acid (pDNA/mRNA) with Opti-MEM.
    c) Slowly add the diluted BAPtofect™-25 to the Opti-MEM containing Nucleic Acid (pDNA/mRNA).
    d) Let stand for 15-30 min at room temperature**.
5. Add Nucleic acid- BAPtofect™-25 complex to cells in Opti-MEM media.
6. Incubate for 2-6 h and then replace with serum growth media.
7. Incubate for the desired end point prior to analysis.

*Best results are seen with sub-confluent cultures. Since each cell type is different, experimentation with different ratios within this range is recommended.

** Incubation temperature time can vary depending upon the system. Nucleic acid - BAPtofect™-25 complexes have been known to stabilize at temperatures ranging from 4 – 55 °C.

Table 1. BAPtofect™-25 volumes required to generate different complexation ratios for 100 ng of nucleic acid.

pDNA (ng)	BAPtofect™-25 (µL) 1 µg/µL	pDNA: BAPtofect™-25 (wt:wt)
100	2	20
100	1	10
100	0.5	5

Note: BAPtofect™-25 volumes should be adjusted based on the quantity of nucleic acid that is to be transfected. For most *in vitro* cell culture based applications, excellent transfection rates can be achieved with Nucleic Acid: BAPtofect™-25 ratios from 1:5 to 1:20.

Table 2. Example of a transfection carried out in HEK293-T cells with plasmid DNA using an 8 well (0.7cm^2 culture area) removable polystyrene media chamber on a glass microscope slide.

Culture Vessel	Media Volume	pDNA Dilution	BAPtofect™-25 Dilution	Transfection Complex Volume	Final Volume
8 well	200 µL	500 ng in 20 µL Opti-MEM media	2.5 µL in 20 µL Opti-MEM media	40 µL	240 µL

Note: The existing procedure has been optimized for *in vitro* transfection in mammalian tissue culture. Nucleic Acid - BAPtofect™-25 complexation can be achieved in water and a variety of buffer conditions for application in animal, plant, and fungal systems. We urge users to optimize complexation ratios and buffer conditions for their specific applications.

Solutions with high concentrations of strong divalent cations such as calcium and magnesium, as well ionic phosphates, might interact with the nucleic acid and affect their complexation with BAPtofect™-25 and could also result in aggregation.

Support Available M-F 8:00 AM - 5:00 PM US CST
support@phoreusbiotech.com
www.phoreusbiotech.com