BAPtofect™-25 Reagent Protocol

Kit Contents

Item #B25005 Size: 0.5 mg (blue cap)
Item #B25010 Size: 1.0 mg (blue cap)
Item #B25050 Size: 5 x 1.0 mg (violet cap)
Item #B25100 Size: 10 x 1.0 mg (violet cap)

Contains both of the following:

Item #P00100 1 mg $CaCl_2$ dried (green cap)
Item #P00200 2.0 mL of nuclease-free H_2O (clear cap)

Storage
Store at room temperature (dried or hydrated).
BAPCs with fluorescent dye must be stored in the dark.

Note: The critical step for transfecting successfully with this kit is determined by finding the best N/P ratio between the positive charges (**N** for Nitrogen) on the BAPtofect carrier and the negative charges (**P** for Phosphate) on the nucleic acid you are trying to deliver. Depending on the kit you received, #B25005 or #B25010, they will contain a total of 3.3×10^{17} or 6.6×10^{17} positive charges, respectively. The number of negative charges per 100 ng of nucleic acid will depend on whether it contains RNA or DNA. There are 1.8×10^{14} negative charges in 100 ng of RNA and 1.7×10^{14} negative charges in 100 ng of DNA. Since you are working with weights, it does not matter if the nucleic acid is single or double stranded.

BAPtofect-25™ Procedure Details

Prepare Stock Solutions

1. Dilute 0.5/1.0 mg of BAPC containing $3.3/6.6 \times 10^{17}$ positive charges stock solution in 50 µL of water to yield 6.6×10^{15}/ 1.3×10^{16} positively charged amino groups (N) per µL.
2. Dilute $CaCl_2$ with 1 mL nuclease-free H_2O to yield 10 mM $CaCl_2$ solution.
3. Prepare nucleic acid solution as salt free solution in sterile water.
4. Combine appropriate volume of BAPCs from 50 µL stock solution with salt free nucleic acid solution.

    a. See table below for sample volumes of BAPCs needed to create different N/P rations for 100 ng of nucleic acid. If you are using a different amount of a nucleic acid, you will need to adjust the volume of the BAPCs. This calculation can be made using a simple ratio equation: BAPC volume for a given N/P ratio divide by 100 ng = new BAPC volume/your nucleic acid nanogram amount. Most applications can achieve excellent transfection rates with N/P ratios of +2 to +20.

5. Alternatively, BAPC aliquots can be dried prior to adding DNA solution to BAPC in order to achieve maximum concentration during BAPC / nucleic acid complexation.

Table 1. BAPC volumes required to generate different N/P Ratios using a standard 100 ng weight of nucleic acid with either the 0.5/1.0 mg kit*. This value will change relative to the number of nanograms of nucleic acid used per experiment.

N/P	µL BAPC	ng of DNA	expts per kit*
20	5/2.5	100	10/20
15	3.8/1.9	100	13/27
10	2.5/1.3	100	20/40
5	1.25/.65	100	40/80
2	0.5/.25	100	100/200

6. Vortex mixture and allow at least 30 minutes for complex formation.
7. Add 1 µL $CaCl_2$ stock solution to the BAPC nucleic acid mixture.
8. Incubate mixture for another 30-60 minutes.
9. Add to cells and incubate for 4 hours, replace media and check after 48 hours.

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

Note: Purchased nucleic acid solutions should be requested as "desalted." Nucleic acid molecules are highly charged and cations, such as sodium and magnesium ions, will neutralize the negative charges and reduce the repulsive interactions between the phosphates. High salt concentrations may result in BAPC / nucleic acid complex aggregation.