BAPtofect™-25 Reagent Protocol

Kit Contents

Item #B25005 Size: 0.5 mg (blue cap)
Item #B25010 Size: 1.0 mg (2 x 0.5 mg vials - 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).
BAPC with fluorescent dye must always be stored in the dark and following hydration at 0° C.

Note: The critical step for successfully transfecting 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 being delivered. Depending on the kit received, #B25005 or #B25010, it will contain an available number of 3.6×10^{16} or 7.2×10^{16} 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. When 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 $3.6/7.2 \times 10^{16}$ positive charges stock solution in 500 µL of water to yield 7.2×10^{14}/ 1.4×10^{15} available 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 BAPC from 500 µL stock solution with salt-free nucleic acid solution.

    a. See table below for sample volumes of BAPC needed to create different N/P ratios for 100 ng of nucleic acid. If using a different amount of a nucleic acid, adjust the volume of the BAPC. This calculation can be made using a simple ratio equation: BAPC volume for a given N/P ratio divided by 100 ng = new BAPC volume/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. This value will change relative to the number of nanograms of nucleic acid used per experiment.

N/P	µL BAPC		ng of DNA	Experiments Per Kit	
	0.5 mg	1.0 mg		0.5 mg	1.0 mg
20	23.4	11.7	100	21	41
15	17.6	8.8	100	27	55
10	11.7	5.9	100	41	83
5	5.9	3.0	100	83	166
2	2.4	1.2	100	208	416

6. Vortex mixture and allow 5 minutes for complex formation.
7. Add 5 µL $CaCl_2$ stock solution per 45 µL volume of the BAPC nucleic acid mixture.
8. Vortex mixture and allow 30 minutes for final binding.
9. Add to cells and incubate for at least 48 hours. It is not necessary to replace media.
10. Check the transfected cells for results.

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 in solutions with highly charged 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.