Insect, Oral dsRNA Protocol for BAPtofect™-25

Kit Contents

Item #B25005 Size: 0.5 mg (**Blue cap**)
Item #B25010 Size: 1 mg (2 x 0.5 mg vials – **Blue cap**)
Item #B25050 Size: 5 x 1 mg (5 x 1 mg vials – **Violet cap**)
Item #B25100 Size: 10 X 1 mg (10 x 1 mg vials – **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 BAPtofect™-25 at room temperature (dried or hydrated).
BAPC with fluorescent dye added must always be stored in the dark.
Green dyed (Atto 488) BAPC should be used immediately following hydration for best fluorescence results.

BAPtofect™-25 Procedure Details

Prepare Stock Solutions:

1. Rehydrate BAPtofect-25 PFAA tube. Add 200 µL (1 mg vial) or 100 µL (0.5 mg vial) nuclease free water to the BAPtofect-25 tube resulting in a 5 µg/µL stock solution and let stand for 20 minutes.

2. Rehydrate stock $CaCl_2$. Add 1 mL of nuclease free water to get 10 mM $CaCl_2$ solution.

3. Dissolve 1 µg of salt-free target dsRNA in 10 µL of RNase-free water to a final concentration of 0.1 µg/µL

4. Add 10 µL of the dsRNA solution dropwise to 10 µL of 5 µg/µL BAPtofect-25 PFAA stock solution and incubate at room temperature for 10 minutes.

5. Vortex for 30 seconds and let stand at room temperature for 20 minutes for binding.

6. For the final binding, add 10 µL of 10mM $CaCl_2$ stock and incubate for another 10-minutes.
7. Dilute this solution to 100 µL with artificial insect (Akey-Beck, 1971) diet ensuring 1mM final $CaCl_2$ concentration.

8. This suspension can be entrapped between two layers of parafilm creating a synthetic phloem sac from which aphids can be fed.

9. For insects treated with lesser amounts of anti-gene dsRNA, BAPC/nucleotide complexes prepared above were diluted 10x and 100x with Akey-Beck diet.

Note: Since each insect type is different, experimenting with different ratios may be necessary. This protocol describes a 50:1 ratio of BAPtofect-25 to dsRNA, however, effective delivery has been found within the range of 20:1 up to 100:1 depending on insect species and dsRNA.

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, may neutralize the negative charges and reduce the repulsive interactions between the phosphates. High salt concentrations may result in BAPC/nucleic acid complex aggregation.

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