

Insect CRISPR-Cas9 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 CaCl₂ dried (**Green cap**)
Item #P00200 2.0 mL of nuclease-free H₂O (**Clear cap**)

Storage

Store 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 tube. Add 100 µL (1 mg kit) or 50 µL (0.5 mg kit) nuclease free water to the BAPtofect-25 tube resulting in a 10 µg/µL stock solution and let stand for 20 minutes.
2. Rehydrate stock CaCl₂. Add 96.2 µL of nuclease free water to 1 mg CaCl₂ to get 10.39 µg/µL CaCl₂ solution.
3. Add 0.5 µl 100 µM of your crRNA #1 (100 ng/µL stock), and 0.5 µl 100 µM TRacrRNA, to 99 µL nuclease-free water. Incubate on ice for 30 minutes.
 - Final concentration of 100 ng/µL of each crRNA and TRacrRNA.
4. Add 2.6 µL of 10 µg/µL BAPtofect-25 stock solution to the mixture which is 26 µg of BAPtofect-25
5. Incubate for 30 minutes at 4° C ; *vortex 30 seconds every 10 minutes*. This is *Solution A*

- If using multiple crRNA repeat steps 3-5 with each crRNA to generate *Solutions B, etc.*
6. Aliquot 100 μL of Solution A or (50 μL of Solution A and 50 μL of Solution B) and add into this solution 10 μL 2.5 μM Cas9 protein. Vortex for 30 seconds and keep at room temperature for 20 minutes.
 7. Add 1 μL of 10.39 $\mu\text{g} / \mu\text{L}$ CaCl_2 solution. Vortex for 2-3 minutes and let stand at room temperature for 20 minutes to generate a final 111 μL volume solution containing the following:
 - 0.450 μM crRNA #1
 - Or 0.225 μM crRNA #1 plus 0.225 μM crRNA #2
 - 0.450 μM TRacrRNA
 - 0.23 $\mu\text{g} / \mu\text{l}$ of BAPtofect-25 PFAA (26 μg BAPtofect-25 in total)
 - 0.225 μM Cas9 protein
 - 93.6 ng / μl of CaCl_2 solution
 8. Inject 2.5 μL * of this insect solution into 4th instar nymphs, 5th instar nymphs or pupae (for insects with body size 2mm or less). Inject ventrally to the side, off-center of midline (avoid ventral nerve chord) along the abdomen using a nanoliter injector or similar device.
***Increase injection volume for larger insects like leafhoppers/planthoppers to 4 μL . For insects such as beetle grubs or lepidopteran caterpillars (both pupae and adults), use 6-10 μL .**
***Decrease injection volume for smaller insects such as mealy bugs (inject 0.6 μL)**
 9. Solution will be stable at room temperature for up to 1 hour. If not utilizing immediately, store suspension on ice for future use. We do not recommend freeze/thawing more than once as it can degrade Cas9 protein.

Support Available M-F 8:00 AM - 5:00 PM US Central

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