ArborEasy® DNA isolation Kit

Protocol ©

Preparation of Extraction Buffer Mix:

Mix 150 µl of Buffer B (mix well by vortexing prior to use); 700µl of Buffer A; 480µl of chloroform and 20µl of Iso-amyl alcohol and pre-heat at 65^0C for 30 minutes in water bath

PROCEDURE:

1. Grind 100 mg tissue sample in mortar & pestle using liquid Nitrogen to a very fine powder.
2. Immediately transfer the ground sample to the pre-heated (65^0C) extraction buffer mix.
3. Vortex for 45 sec thoroughly and centrifuge at 14,000 rpm for 15 min at room temperature.
4. Transfer the supernatant (~ 600 µl) and add equal volume of chilled Iso-propanol.
5. Add 1.5 volume of Binding buffer (**without ethanol**) and mix gently by pipetting*.
6. Add 750µl of the mixture to the filter column and centrifuge at 12000 rpm for 2 min and discard the flow through.
7. Repeat step 6 for the remaining sample mixture.
8. Add 700 µl of Wash buffer W1 (**350 µl W1 + 350 µl of absolute ethanol**) to the filter column and centrifuge at 12000 rpm for 1 min and discard the flow through**.
9. Add 750 µl of Wash buffer W2 (**150 µl W2 + 600 µl of absolute ethanol**) to the filter column and centrifuge at 12000 rpm for 1 min and discard the flow through**.
10. Repeat the step 9.
11. Centrifuge the empty Filter column for 3 min to remove ethanol. Make sure that the column is completely dry prior to adding elution buffer.
12. Take a fresh vial and add 50 µl of the pre-heated (at 65^0C) elution buffer and incubate for 3 min at room temperature and centrifuge at 12000 rpm for 2 min and collect the eluate. This step can be repeated for additional 50 µl of elution buffer.

Note:

* ***It is 1.5 volume of supernatant and not total reaction mix with isopropanol.***

** ***Ethanol is not provided in the kit.***

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