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# F A Q :
# PlantOpsis™ DirectPCR-A

1. **How is DirectPCR-A different from DirectPCR-B?**
    a. Both products mediate direct PCR from plant samples, but through different means and each has a distinct formulation. The composition of one may result in better amplification of target genes for desired applications compared to the other. It's unlikely that a single direct PCR reagent will be optimal for all situations, since our customers and research partners use our DirectPCR products with numerous types of plant samples and for diverse applications.

    We strive to provide solutions that meet our customers' needs. We are happy to custom formulate a DirectPCR product for your specific needs if currently available products are not optimal (at no additional cost).

2. **Can DirectPCR-A be used to amplify diverse specimen types?**
    a. Yes. DirectPCR-A can potentially be used to amplify gene targets from plant samples, along with numerous pathogens associated with plants. The ability of the reagent to mediate direct PCR for specific applications should be verified by the end user. We offer 1 mL vials for these exploratory studies.

3. **How much processed sample should I add to my PCR mixture?**
    a. This greatly depends on your sample and means of detecting nucleic acid amplification. For example, 40% of your PCR mixture can be sample if you are testing plants with low levels of PCR inhibitors through quantitative PCR. Alternatively, you may want to only use a PCR mixture with 5~10% sample if you are detecting amplification through colorimetric means with LAMP.

4. **Is DirectPCR-A compatible with automation?**
    a. Yes. Follow the same protocol with automated systems.

5. **What's the lowest volume of DirectPCR-A I can use with my leaf punch?**
    a. Validation studies were performed using 50~100 µL of reagent and leaf punches with a diameter of 4~6 mm. The use of alternative reaction volumes or sample sizes needs to be validated by the user. However, you might be able to use 25 µL / sample for some applications.

6. **Do I need to change my Ct thresholds when using DirectPCR-A compared to RNA / DNA extraction?**
    a. You may need to increase your Ct thresholds depending on your specific application and needs.

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7. **Can DirectPCR-A be used for endpoint PCR, isothermal PCR, and quantitative PCR?**
    a. The components of DirectPCR-A are compatible with all types of PCR applications. The selection and quantity of your plant sample plays the greatest role in determining success with DirectPCR-A.

8. **I'll like to use DirectPCR-A in a manner that's different than intended. How should I proceed?**
    a. The recommended protocol is a good starting point for exploring unintended applications. You can vary the ration of reagent to sample, heating, and primer / probe selection. The R&D team at Entopsis is always ready to assist with new applications. Send us your needs (info@entopsis.com) and we may already have a solution for you.

9. **For how long is DirectPCR-A stable if properly maintained?**
    a. If stored properly, 18 months from date of manufacture.

10. **Can DNase be added to DirectPCR-A processed samples?**
    a. Yes.

11. **Is there a benefit to heating the sample + reagent mixture at 95˚C for longer than recommended?**
    a. Heating at 95˚C for longer than recommended may be beneficial if suboptimal results are observed, especially with difficult to lyse microorganisms. Alternatively, if the heating device is not at 95˚C when the sample is placed or if thin walled tubes / plates are not used, then a prolonged heating step is beneficial. Heating samples a bit longer than recommended will not negatively affect your results for most applications.

12. **I cannot heat my sample + reagent to 95˚C. Can I heat it at a lower temperature but for a longer period?**
    a. Yes. Heating at 80~85˚C for 15~25 minutes offers comparable results to heating at 95˚C for many applications.

13. **The product looks hazy, is this normal?**
    a. Yes. This is normal. Be sure DirectPCR-A is homogenized before use.

14. **How do I homogenize DirectPCR-A before use?**
    a. Simply invert the bottle a few times without creating too many bubbles. You can also pipette up / down a few times to ensure complete mixing.

15. **Can I process samples with DirectPCR-A but perform PCR at a later point in time?**
    a. Processed samples may remain at room temperature for ~8 hours before performing PCR and longer storage may be possible at 4˚C ~ -80˚C. Users seeking to store processed samples should keep in mind that the stability of your RNA may depend on your sample type, how you store the sample and period of storage. Please confirm the stability of your RNA with a properly

controlled study if processed samples are not going to be used for PCR studies within a few hours after processing. DirectPCR-A processed samples are expected, but not yet fully validated, to be stable if stored at -80˚C for a few months.

16. **RNA extraction procedures result in the lost of some fragments and enrichment of others, thus producing vendor specific bias. Does DirectPCR-A also have this problem?**
    a. No, because DirectPCR-A does not require the capture and release of RNA. As such, you are left with a complete RNA profile. Studies are required to compare DirectPCR-A to extraction protocols concerning this point. There's a body of literature demonstrating that RNA extraction protocols result in different levels of small RNA fragments (e.g., miRNA) and thereby introduce bias into your data. This problem is specific to RNA extraction procedures because each vendor's nucleic acid capture device (e.g., column, beads, etc.) has inherent affinities for given targets; thus, bias is unavoidable.