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F A Q :
PCR*opsis*™ Reagent RVD-RT

1. **Does Reagent RVD-RT need to be mixed with Activator before processing samples?**
    a. Yes. Reagent RVD-RT needs to be mixed with Activator for optimal performance. **Activator is included when the term "Reagent RVD-RT" or "activated Reagent RVD-RT" are used, unless otherwise indicated.**

2. **For how long is the Reagent RVD-RT-Activator mixture stable?**
    a. The mixture should be used within 4 hours of preparing if stored at room temperature or within 24 hours of preparing if stored at 4˚C.

3. **Can Reagent RVD-RT be used to amplify diverse specimen types?**
    a. Yes. Reagent RVD-RT can be used to amplify gene targets from viruses and mammalian cells from saliva or swab samples in compatible transport mediums. Some, but not all bacteria may not be thoroughly lysed with Reagent RVD-RT at room temperature, and may require heating of the 1:1 reagent : sample mixture to 60˚C ~ 95˚C if sub-optimal lysis is observed. Pure saliva (i.e., not in transport mediums) is compatible with Reagent RVD-RT.

4. **Is Reagent RVD-RT compatible with automation?**
    a. Yes. Follow the same Reagent RVD-RT protocol with automated systems.

5. **What's the lowest volume of Reagent RVD-RT and sample I can use?**
    a. Validation studies with Reagent RVD-RT used 20 µL of reagent and 20 µL of sample. The use of alternative reaction volumes needs to be validated by the user.

6. **What's the optimal ratio of Reagent RVD-RT to sample?**
    a. The optimal ratio is 1:1 for most saliva, urine or swab specimens in transport mediums.

7. **If needed, how do you recommend samples be heated once mixed with Reagent RVD-RT?**
    a. Samples mixed 1:1 with the reagent should be heated with a temperature controlled thermal cycler or heating block. Make sure the heating device reaches the desired temperature before applying samples to the heating device. For best results, sample / reagent mixtures in 96-well plates and sealed with a plate sealer should be heated in a manner that prevents the evaporation of material (e.g., thermal cycler or heating block with a top lid).

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8. **Does processing samples with Reagent RVD-RT allow for the detection of human nucleic acids often found in clinical swab samples?**
    a. Yes. Human RNA / DNA can be detected from Reagent RVD-RT processed samples.

9. **Is Reagent RVD-RT guaranteed to work?**
    a. Yes. Reagent RVD-RT is guaranteed when used as intended with select viral and mammalian specimens, but may not produce optimal results with all bacterial or fungal species. It's the user's responsibility to confirm the suitability of the reagent for unintended applications with a proper validation study. The research team at Entopsis is here to help.

10. **Do I need to change my Ct thresholds when using Reagent RVD-RT compared to RNA / DNA extraction?**
    a. Yes. You may need to increase your RT-qPCR thresholds when using Reagent RVD-RT. For example, some users have set their Ct thresholds for RT-qPCR using Reagent RVD-RT to ~40 when amplifying N1 and N2 regions of SARS-CoV-2.

11. **I'll like to use Reagent RVD-RT in a manner that's different than intended. How should I proceed?**
    a. The suitability of Reagent RVD-RT for unintended applications has not been validated. A proper validation study is necessary before Reagent RVD-RT can be used for unintended in vitro diagnostic applications. For such validation studies, you should start by mixing Reagent RVD-RT 1:1 with your sample; this may work for most applications where the concentration of RT-qPCR inhibitors is not high. If desired results are not observed, then add more or less reagent relative to your sample (e.g., 2.5:1, 5:1, 10:1) and/or heat at 95˚C for a longer period of time. The research team at Entopsis is here to help if you have other related questions.

12. **Does Reagent RVD-RT work with all microbial transport mediums?**
    a. Reagent RVD-RT has been shown to function properly with microorganisms collected in various universal viral transport systems, PBS, CDC VTM, WHO VTM, 100% saliva and centrifuged urine. Reagent RVD-RT may replace RNA / DNA extraction when other transport medias or samples are used, but the user needs to confirm the suitability of this product in such cases with a pilot study. The research team at Entopsis is here to help.
    NOTE: Reagent RVD-RT is not expected to function properly with transport medias containing toxic guanidinium thiocyanate / guanidinium isothiocyanate, alcohols, or any component that inhibits the function of reverse transcriptase and / or polymerase.

13. **For how long is Reagent RVD-RT stable if properly maintained?**
    a. Reagent RVD-RT (alone) is stable for 18 months from date of manufacture and Activator is stable for 12 months from date of manufacture.

14. **Can Reagent RVD-RT be used for non-viral applications?**
    a. Yes. Reagent RVD-RT facilitates extraction-free amplification of RNA / DNA gene targets from various specimen types (mostly viruses and mammalian cells). The user should perform a pilot study to confirm the suitability of Reagent RVD-RT for the desired application.

15. **Can Reagent RVD-RT be used for DNA extraction?**
    a. Yes. Reagent RVD-RT facilitates extraction-free amplification of RNA / DNA gene targets.

16. **How long should I heat my sample / reagent mixture at 95˚C for the detection of difficult to lyse bacteria?**
    a. The mixture should be heated for 10~15 minutes to ensure complete lysis and accessibility to nucleic acids.

17. **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 60~85˚C for 20~25 minutes offers comparable results to heating at 95˚C for many applications.

18. **The product looks hazy, is this normal?**
    a. Yes. This is normal. Be sure Reagent RVD-RT is homogenized before use.

19. **I noticed two liquid phases with Reagent RVD-RT, is this normal?**
    a. Yes. The reagent consists of 2 phases, one clear and one hazy. This is noticeable when the product is not mixed.

20. **How do I homogenize Reagent RVD-RT 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.

21. **Can I process samples with Reagent RVD-RT but perform PCR / RT-qPCR at a later point in time?**
    a. For optimal results, the processed sample needs to be used for downstream PCR applications within ~4 hours after the 10-minute incubation.

22. **How is the functionality and sterility of Reagent RVD-RT determined?**
    a. Every lot of Reagent RVD-RT is tested using a stock viral samples. This sample is processed with the reagent and the RNA / DNA is extracted using Qiagen's QIAamp RNA / DNA kit, and RT-qPCR performed. Ct values for both methods must be within 5 Ct of each other for the lot to pass. Sterility is confirmed by placing the reagent onto blood agar plates and observe growth after 72 hours at 37˚C. The lot passes our quality control criteria if these tests are satisfactory.

23. **RNA extraction procedures result in the lost of some fragments and enrichment of others, thus producing vendor specific bias. Does Reagent RVD-RT also have this problem?**
    a. No, because Reagent RVD-RT does not require the capture and release of RNA. As such, you are left with a complete RNA profile. Studies are required to compare Reagent RVD-RT 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.