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F A Q :
PCR*opsis*™ Reagent RVD with RVD Enhancer

1. **Can Reagent RVD with RVD Enhancer be used to amplify diverse specimen types?**
    a. Yes. Reagent RVD with RVD Enhancer can be used to amplify gene targets from viruses, bacteria and mammalian cells from saliva, urine or swab samples in compatible transport mediums. Pure saliva or urine (i.e., not in transport mediums) is compatible with Reagent RVD with RVD Enhancer. NOTE: Reagent RVD with RVD Enhancer performs well with SARS-CoV-2 gene targets, N1, N2, E and NJ, but not with Orf1a.

2. **Is Reagent RVD with RVD Enhancer compatible with automation?**
    a. Yes. Follow the same Reagent RVD with RVD Enhancer protocol with automated systems.

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

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

5. **How do you recommend samples be heated once mixed with Reagent RVD with RVD Enhancer?**
    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).

6. **Does processing samples with Reagent RVD with RVD Enhancer 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 with RVD Enhancer processed samples. RNA / DNA from human epithelial cells are accessible after ~5 minutes of heating your reagent / sample mixture at 95˚C.

7. **Is Reagent RVD with RVD Enhancer guaranteed to work?**
    a. Yes. Reagent RVD with RVD Enhancer is guaranteed when used as intended. 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.

8. **Do I need to change my Ct thresholds when using Reagent RVD with RVD Enhancer compared to RNA / DNA extraction?**
    a. Not usually. For most sample types and gene targets, you are expected to observe comparable Ct values between samples processed with our reagent and RNA / DNA extraction that doesn't concentrate nucleic acids. You may consider extending the Ct threshold to 40~45 cycles to improve assay sensitivity for some applications.

9. **I'll like to use Reagent RVD with RVD Enhancer in a manner that's different than intended. How should I proceed?**
    a. The suitability of Reagent RVD with RVD Enhancer for unintended applications has not been validated. A proper validation study is necessary before Reagent RVD with RVD Enhancer can be used for unintended in vitro diagnostic applications. For such validation studies, you should start by mixing Reagent RVD with RVD Enhancer 1:1 with your sample; this may work for some 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.

10. **Does Reagent RVD with RVD Enhancer work with all microbial transport mediums?**
    a. Reagent RVD with RVD Enhancer 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 with RVD Enhancer 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 with RVD Enhancer is not expected to function properly with transport medias containing toxic guanidinium thiocyanate / guanidinium isothiocyanate.

11. **For how long is Reagent RVD with RVD Enhancer stable if properly maintained?**
    a. If stored properly, 18 months from date of manufacture.

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

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

14. **Can DNase be added to Reagent RVD with RVD Enhancer processed samples?**
    a. Yes.

15. **How long should I heat my sample / reagent mixture at 95˚C?**
    a. Mammalian: 5 minutes
    Viruses: 10 minutes
    Bacteria: 15 minutes
    - Select the longer heating time when working with mixed cultures.
    - Human cells found in nasopharyngeal and oropharyngeal swab samples are easily lysed after ~5 minutes at 95˚C.

16. **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.

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 80~85˚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 with RVD Enhancer is homogenized before use.

19. **I noticed two liquid phases with Reagent RVD with RVD Enhancer, 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 with RVD Enhancer 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 with RVD Enhancer but perform RT-qPCR at a later point in time?**
    a. Processed samples may remain at room temperature for ~8 hours before performing RT-qPCR 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 RT-qPCR studies within a few hours after processing. Reagent RVD with RVD Enhancer processed samples are expected, but not yet fully validated, to be stable if stored at -80˚C for a few months.

22. **How is the functionality and sterility of Reagent RVD with RVD Enhancer determined?**
    a. Every lot of Reagent RVD with RVD Enhancer is tested using a stock virus or bacterial sample. 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 with RVD Enhancer also have this problem?**
    a. No, because Reagent RVD with RVD Enhancer 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 with RVD enhancer 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.