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# F A Q : PCR*opsis*™ Reagent SRVD

1. **Is Reagent SRVD compatible with automation?**
    a. Yes. Follow the same Reagent SRVD protocol with automated systems.

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

3. **What's the optimal ratio of Reagent SRVD to sample?**
    a. The optimal ratio is 1:1 for most specimens in saliva.

4. **How do you recommend samples be heated once mixed with Reagent SRVD?**
    a. Samples mixed 1:1 with Reagent SRVD should be heated with a temperature controlled heating block or thermal cycler. Make sure the heating device reaches the desired temperature before applying samples to the heating device.

5. **Does processing samples with Reagent SRVD allow for the detection of human nucleic acids often found in clinical swab samples?**
    a. Yes. Human RNA / DNA can be detected from Reagent SRVD processed samples. RNA / DNA from human epithelial cells are accessible after 5 minutes of heating your Reagent SRVD / sample mixture at 95˚C.

6. **Is Reagent SRVD guaranteed to work?**
    a. Yes. Reagent SRVD is guaranteed when used as intended. It's the user's responsibility to confirm the suitability of Reagent SRVD for unintended applications with a proper validation study. The research team at Entopsis is here to help.

7. **Do I need to change my Ct thresholds when using Reagent SRVD compared to RNA / DNA extraction?**
    a. Yes. You will usually need to increase your RT-qPCR thresholds when using Reagent SRVD. For example, other Reagent SRVD users have set their Ct thresholds for RT-qPCR using Reagent SRVD to ~45 when amplifying N1 and N2 regions of SARS-CoV-2.

8. **I'll like to use Reagent SRVD in a manner that's different than intended. How should I proceed?**
    a. The suitability of Reagent SRVD for unintended applications has not been validated. A proper validation study is necessary before Reagent SRVD can be used for unintended in vitro diagnostic applications. For such validation studies,

you should start by mixing Reagent SRVD 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 Reagent SRVD 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.

9. **Does Reagent SRVD work with all saliva transport mediums?**
    a. Reagent SRVD is likely to work with various non-inactivating transport mediums, however the user should confirm the compatibility of their transport medium. Most studies to date have used 100% saliva, without the use of transport mediums. Reagent SRVD is not expected to function properly with transport medias containing toxic guanidinium thiocyanate / guanidinium isothiocyanate.

10. **For how long is Reagent SRVD stable if properly maintained?**
    a. If stored properly, 18 months from date of manufacture.

11. **Can Reagent SRVD be used for non-viral applications?**
    a. Yes. Reagent SRVD facilitates extraction-free amplification of RNA / DNA gene targets from various microorganisms (viruses, bacteria and fungi). The user should perform a pilot study to confirm the suitability of Reagent SRVD for the desired application.

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

13. **Can DNase be added to Reagent SRVD processed samples?**
    a. Yes.

14. **How long should I heat my sample / Reagent SRVD 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 samples are easily lysed after 5 minutes at 95˚C.

15. **Is there a benefit to heating the sample + Reagent SRVD 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.

16. **I cannot heat my sample + Reagent SRVD 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.

17. **The product looks hazy, is this normal?**
    a. Yes. This is normal. Be sure Reagent SRVD is homogenized before use.

18. **I noticed two liquid phases with Reagent SRVD, is this normal?**
    a. Yes. Reagent SRVD consists of 2 phases, one clear and one hazy. This is noticeable when the product is not mixed.

19. **How do I homogenize Reagent SRVD 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.

20. **Can I process samples with Reagent SRVD 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. Users seeking to store Reagent SRVD processed samples should keep in mind that the stability of your RNA may depend on your sample type, how you store the sample and the time frame following processing with Reagent SRVD. 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 immediately after processing. Reagent SRVD processed samples are expected, but not yet validated, to be stable if stored at -80˚C for a few months.

21. **How is the functionality and sterility of Reagent SRVD determined?**
    a. Every lot of Reagent SRVD is tested using a stock virus or bacterial sample. This sample is processed with Reagent SRVD 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 Reagent SRVD 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.

22. **DNA / RNA extraction procedures result in the lost of some fragments and enrichment of others, thus producing vendor specific bias. Does Reagent SRVD also have this problem?**
    a. No, because Reagent SRVD 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 SRVD 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.