v.20210201

F A Q : PCR*opsis*™ Reagent RVD-E

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

2. **What type of specimens can be amplified with Reagent RVD-E?**
    a. To date, various gene targets from viruses, bacteria (both gram positive and negative) and human cells have been amplified with Reagent RVD-E. These specimens have been amplified at low concentrations (i.e., <100 cfu / mL). Difficult to lyse specimens (i.e., fungi) may be detected at higher concentrations and may require a longer heating period at 95˚C. The user should perform a pilot study with desired specimens to ensure Reagent RVD-E is suitable for the desired application.

3. **What's the lowest volume of Reagent RVD-E I can use?**
    a. Validation studies with Reagent RVD-E used 200 µL of reagent in a V- or U-bottom 10 mL transport tube. The use of alternative reaction volumes needs to be validated by the user.

4. **How do you recommend samples be heated once mixed with Reagent RVD-E?**
    a. Samples mixed with Reagent RVD-E should be heated in 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 RVD-E 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-E processed samples. RNA / DNA from human epithelial cells are accessible after 5 minutes of heating your Reagent RVD-E / sample mixture at 95˚C.

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

7. **I'll like to use Reagent RVD-E in a manner that's different than intended. How should I proceed?**
    a. The suitability of Reagent RVD-E for unintended applications may not have been validated. A proper validation study is necessary before Reagent RVD-E can be used for unintended in vitro diagnostic applications. The research team at Entopsis is here to help if you have other related questions.

8. **For how long is Reagent RVD-E stable if properly stored?**
    a. If stored properly, 18 months from date of manufacture.

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

10. **Can DNase be added to Reagent RVD-E processed samples?**
    a. Yes.

11. **How long should I heat my sample / Reagent RVD-E 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, oropharyngeal and buccal swab samples are easily lysed after 5 minutes at 95˚C.

12. **Is there a benefit to heating the sample + Reagent RVD-E 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 (e.g., fungi). 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.

13. **I cannot heat my sample + Reagent RVD-E 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.

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

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

16. **How do I homogenize Reagent RVD-E 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.

17. **Can I process samples with Reagent RVD-E 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 RVD-E 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 RVD-E. 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 RVD-E processed samples are expected, but not yet validated, to be stable if stored at -80˚C for a few months.

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

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