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# F A Q : PCRopsis™ Reagent Buccal

1. **Is Reagent Buccal compatible with automation?**
  - a. Yes. Follow the same Reagent Buccal protocol with automated systems.
2. **What type of specimens can be amplified with Reagent Buccal?**
  - a. This product is specifically engineered for extraction-free PCR amplification of human cheek cells absorbed onto buccal swabs transported dry (i.e., not in transport mediums). The user should perform pilot studies with alternative specimens or collection approaches to ensure Reagent Buccal is suitable for the desired application.
3. **What's the lowest volume of Reagent Buccal I can use?**
  - a. Validation studies with Reagent Buccal 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 Buccal?**
  - a. Samples mixed with Reagent Buccal 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. **Is Reagent Buccal guaranteed to work?**
  - a. Yes. Reagent Buccal is guaranteed when used as intended. It's the user's responsibility to confirm the suitability of Reagent Buccal for unintended applications with a proper validation study. The research team at Entopsis is here to help.
6. **I'll like to use Reagent Buccal in a manner that's different than intended. How should I proceed?**
  - a. The suitability of Reagent Buccal for unintended applications may not have been validated. A proper validation study is necessary before Reagent Buccal can be used for unintended in vitro diagnostic applications. The research team at Entopsis is here to help. Please contact us at [info@entopsis.com](mailto:info@entopsis.com) with your desired application and one of our scientists will try to help.
7. **For how long is Reagent Buccal stable if properly stored?**
  - a. If stored properly, 18 months from date of manufacture.
8. **Can DNase be added to Reagent Buccal processed samples?**
  - a. Yes.



- 9. Is there a benefit to heating the sample + Reagent Buccal mixture at 95°C for longer than recommended?**
- Heating at 95°C for longer than recommended may be beneficial if suboptimal results are observed. 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.
- 10. I cannot heat my sample + Reagent Buccal to 95°C. Can I heat it at a lower temperature but for a longer period?**
- Yes. Heating at 80~85°C for 20~25 minutes offers comparable results to heating at 95°C for many applications.
- 11. The product looks hazy, is this normal?**
- Yes. This is normal. Be sure Reagent Buccal is homogenized before use.
- 12. I noticed two liquid phases with Reagent Buccal, is this normal?**
- Yes. Reagent Buccal consists of 2 phases, one clear and one hazy. This is noticeable when the product is not mixed.
- 13. How do I homogenize Reagent Buccal before use?**
- 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.
- 14. Can I process samples with Reagent Buccal but perform PCR at a later point in time?**
- Processed samples may remain at room temperature for ~8 hours before performing PCR and longer storage may be possible. Users seeking to store Reagent Buccal 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 Buccal. 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 Buccal processed samples are expected, but not yet validated, to be stable if stored at -80°C for a few months.
- 15. How is the functionality and sterility of Reagent Buccal determined?**
- Every lot of Reagent Buccal is tested using a stock samples. This sample is processed with Reagent Buccal and qPCR performed. Observed Ct values need to fall within a defined range. Sterility is confirmed by placing Reagent Buccal 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.



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**16. DNA / RNA extraction procedures result in the loss of some fragments and enrichment of others, thus producing vendor specific bias. Does Reagent Buccal also have this problem?**

- a. No, because Reagent Buccal 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 Buccal 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.