**Frequently Asked Questions**

**VersaTaq™ Direct PCR Polymerase [PN 71002]**

**General**

1. Is there anything that should be done prior to starting an experiment with VersaTaq™ Direct PCR Polymerase?
   - Read through the VersaTaq Direct PCR Polymerase long protocol thoroughly and note any sample specific recommendations.
   - Optimization experiments are often required to determine appropriate conditions for a particular sample when performing direct PCR. See question 2 below for recommended optimizations.
   - Contact USB Technical Support for additional troubleshooting tips and recommendations at USBtechsupport@affymetrix.com.

2. What are the recommended steps that can be taken to optimize VersaTaq Direct PCR experiments?
   a. Perform titration experiments with VersaTaq Direct PCR Polymerase, in a range from 0.1-1.0 µl per 25 µl reaction.
   b. Increase the initial denature time at 95°C to 6-10 minutes.
   c. Increase the extension time to 3-4 minutes per kb.
   d. Increase amount of cycles, up to 45 cycles, for low copy targets.
   e. Add less sample to the reaction. Sometimes adding too much sample can inhibit the reaction.
   f. If amplification is not satisfactory, it might be necessary to add enhancers to the reaction. It is recommended to start with adding 1 M Betaine or 10% DMSO. If conditions do not improve, contact USB Technical Support for additional information on enhancers (USBtechsupport@affymetrix.com).

3. Are there recommended control primers that can be used to test direct amplification using VersaTaq Direct PCR Polymerase on my particular samples?
   Control primers can be provided upon request. Please contact USB Technical Support for additional information on control primers (USBtechsupport@affymetrix.com).

4. My genomic DNA controls generate a smear after running the products on a gel, but my direct samples give nice bands. What can be done to improve the gDNA results?
   Typically, 5-10 times less VersaTaq Direct PCR Polymerase (i.e., ~0.05 µl per 25 µl reaction) is required when amplifying controls that do not contain any PCR inhibitors, as too much enzyme can lead to smearing or even inhibit the reaction.

5. How does VersaTaq Direct PCR Polymerase differ from Taq DNA Polymerase?
   VersaTaq Direct PCR Polymerase is a mutant Taq DNA polymerase engineered for direct amplification from complex inhibitory templates. It confers resistance to many potent PCR inhibitors such as those found in blood, tissue (e.g., mouse tail), feces, soil, and other sample types.

6. Is VersaTaq Direct PCR Polymerase a hot start polymerase?
   No, VersaTaq Polymerase has a cold sensitive mutation that mitigates any non-specific amplification during reaction setup on ice. VersaTaq Direct PCR Polymerase does not have a modification that requires activation steps.

7. Is VersaTaq Direct PCR Polymerase suitable to be used in qPCR assays?
   Yes, VersaTaq Polymerase has 5’-3’ exonuclease activity, making it suitable for hydrolysis probe-based detection chemistries. Please contact USB Technical Support (USBtechsupport@affymetrix.com) for more information on direct qPCR applications.

8. Are there any restrictions to the size of the amplicon when using VersaTaq Direct PCR Polymerase?
   Since VersaTaq Polymerase is a mutant of Taq, the amplicon size restrictions are similar to Taq polymerase. The maximum length is highly dependent on the sample type (i.e., targets up to 5 kb from blood, and targets up to 2 kb from tissue).

9. After performing direct PCR with VersaTaq Direct PCR Polymerase, how should I store the PCR products?
   For short term storage (a day), samples can be stored at 4°C. For long term storage, samples can be stored at -20°C.
10. Is VersaTaq Direct PCR Polymerase compatible with standard Taq reaction buffers?
   
   No, VersaTaq Polymerase requires its own reaction buffer that is supplied as a separate tube with the polymerase.

11. Is VersaTaq Direct PCR Polymerase compatible with PCR enhancers such as Betaine and DMSO?
   
   Yes, when amplifying difficult targets (e.g., high GC), 1 M Betaine or 10% DMSO is a general starting point for optimizing VersaTaq Direct PCR Polymerase.

12. What percent GC amplicon targets have been tested with VersaTaq Direct PCR Polymerase?
   
   We have tested up to 80% GC with VersaTaq Polymerase. Addition of 1 M Betaine is typically required for high GC templates.

13. What can be done when Betaine and DMSO do not help with amplifying high GC targets?
   
   Contact USB Technical Support for additional information on enhancers (USBtechsupport@affymetrix.com).

14. Can ExoSAP-IT® reagent be used after VersaTaq Direct PCR Polymerase where Sanger sequencing is the downstream application?
   
   Yes, 50 µl PCR reactions should be used to dilute any inhibitors that may be present. Then, take 5 µl of this PCR product (without Proteinase K treatment) and treat with 2 µl of ExoSAP-IT reagent, following the accompanying ExoSAP-IT protocol.

Blood

15. Can VersaTaq Direct PCR Polymerase be used with previously frozen blood?
   
   Yes, VersaTaq Polymerase can be used with fresh whole blood or frozen whole blood that has been thawed on ice.

16. How should one store blood prior to using VersaTaq Direct PCR Polymerase?
   
   For short term storage, blood can be stored at 4°C (several days). For long term storage, blood should be stored at -80°C.

17. What anticoagulants can VersaTaq Direct PCR Polymerase be used with in blood applications?
   
   VersaTaq Polymerase has been tested in the presence of Na-Citrate, Na-Heparin, and Na-EDTA with exceptional performance.

18. Is VersaTaq Direct PCR Polymerase compatible with blood stored on paper cards?
   
   Yes, VersaTaq Polymerase was extensively tested with Whatman™ FTA™ cards and also works for several types of paper cards including 903.

19. How long can blood be stored on blood cards prior to using them with VersaTaq Direct PCR Polymerase?
   
   We have tested blood stored on blood cards for 1 year with VersaTaq Direct PCR Polymerase and still had robust amplification. According to the respective manufacturer, blood is stable on Whatman FTA cards for several years.

20. I do not have a 2 mm punch available to cut from blood cards. Is there anything else I can use?
   
   If the recommended 2 mm Harris Uni-Core™ Punch is not available, a scalpel or razor blade can be used to cut a small piece (approximately 2 mm wide).

21. What is an acceptable blood concentration range while using VersaTaq Direct PCR Polymerase?
   
   VersaTaq Direct PCR Polymerase shows successful amplifications with as low as 0.5% blood and up to 40% blood concentrations.

Animal tissue (including mouse tail)

22. I am testing the VersaTaq Direct PCR protocol with animal tissue and there seems to be a lot of DNA stuck in the wells. What can I do?
   
   When amplifying DNA directly from tissue, it is required to treat the completed PCR reaction with Proteinase K prior to gel electrophoresis to prevent cell debris and proteins from causing PCR products to get stuck in the wells.
23. What concentration of Proteinase K should be used to treat PCR products from animal tissue prior to gel electrophoresis?

For animal tissue analysis, dilute Proteinase K (PN 76225) 1:10 in 6X DNA Loading Buffer (PN 76715). For example, add 5 µl of Proteinase K into 45 µl 6x DNA Loading Buffer. Then, add 15 µl of DNA Loading Buffer-Proteinase K mix into finished 50 µl PCR reactions (Proteinase K final concentration of 0.2 mg/ml). Mix reactions and incubate for 30-60 minutes at 50°C. Spin tubes and analyze sample by agarose gel electrophoresis.

**Plant tissue**

24. In the Plant Tissue Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, it lists 1 M Betaine in the components table for the PCR reaction. Is Betaine required?

Yes, Betaine is required for any reactions using plant tissue. If Betaine is not available, please use 10% DMSO.

25. In the Plant Tissue Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, it only has a protocol for two types of plant tissue (leaf and seed). If I want to use root, which protocol do I follow?

Follow the direct protocol from the plant leaves section even though both the leaf and seed direct protocols are very similar.

26. In the Plant Tissue Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, it has protocols for both dilution and direct reactions. If sample retention isn’t an issue, which protocol should I use?

Use the direct protocol whenever possible.

**Feces**

27. When using VersaTaq Direct PCR Polymerase with fecal samples, is it possible to add a small fecal sample directly to the PCR reaction and get successful amplification?

No, we recommend following the Fecal Crude Extract Preparation Tutorial for use with VersaTaq Direct PCR Polymerase.

28. In the Fecal Crude Extract Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, it has a section on fecal samples stored in a stabilizer solution. What is meant by stabilizer solution?

Tissue and other types of samples can be harvested and placed into solution for storage without jeopardizing the quality of cellular components for later analysis. A stabilizing solution protects nucleic acids and other cellular components. RNAlater®, DNA/RNA Shield™, RNAssist, and others are considered stabilizing solutions.

29. When using the Fecal Crude Extract Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, why is it important to wash the sample after it was stored in a stabilizer solution?

Stabilizing solutions are highly inhibitory of VersaTaq Direct PCR Polymerase so it is important to get as much of the stabilizing solution out of the reaction as possible.

30. In the Fecal Crude Extract Preparation Tutorial for use with VersaTaq Direct PCR Polymerase, it notes that sonication or a bead beater step might be required with microorganisms such as gram positive bacteria or fungi for more efficient cell lysis. If a lab doesn’t have a sonicator or bead beater, are there other tools that could be use?

Any type of tool that can grind, shear, beat, or shock the cells to disrupt the cells wall will suffice. Some other examples are blender, mortar and pestle, glass, and dounce homogenizers.

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