PureIT ExoZAP PCR CleanUp provides PCR Clean-Up in 5 minutes

PureIT ExoZAP PCR CleanUp offers a fast PCR clean-up method with superior clean-up results compared to other enzymatic PCR clean-up products on the market. Results are obtained in just 5 minutes using a very simple one-step workflow. See figure 1. PureIT ExoZAP PCR CleanUp consists of a heat labile Exonuclease I (HL-ExoI) and a recombinant Shrimp Alkaline Phosphatase (rSAP). Treatment of PCR products with PureIT ExoZAP PCR CleanUp degrades residual primers and dephosphorylates dNTPs in 5 minutes. After enzymatic treatment at 37 °C for minimum 2 minutes, the enzymatic activities of PureIT ExoZAP PCR CleanUp are completely inactivated by heating at 80 °C for minimum 3 minutes. The use of PureIT ExoZAP PCR CleanUp eliminates the need for spin columns, magnetic beads, filtration and gel purifications.

Features

- One-step PCR product clean-up
- 5 min protocol
- No need for spin columns or magnetic beads
- Scalable for different reaction sizes
- Treatment optimizes downstream applications such as DNA sequencing or SNP analysis
- No sample loss

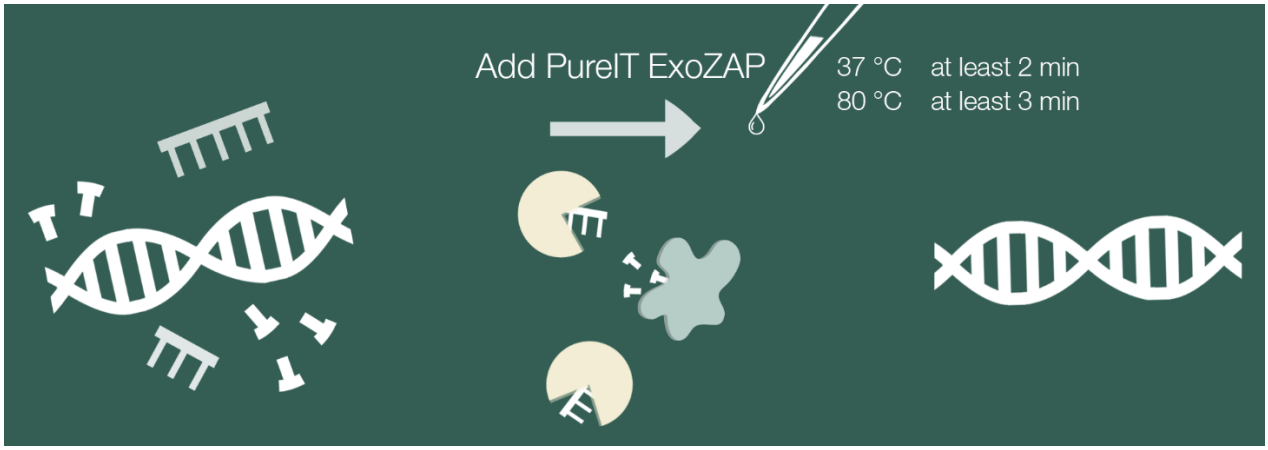

Figure 1. *One-step scheme for PureIT ExoZAP PCR CleanUp. Enzymatic treatment of PCR products with PureIT ExoZAP PCR CleanUp degrades residual primers (ExoI) and dephosphorylates dNTPs (rSAP). After enzymatic treatment at 37 °C for minimum 2 minutes, the enzymatic activities are completely inactivated by heating at 80 °C for minimum 3 minutes.*

Clean-up of PCR products using PureIT ExoZAP PCR CleanUp

This protocol serves as a guideline for the clean-up of 5 µl PCR product using PureIT ExoZAP PCR CleanUp.

1. Take out PureIT ExoZAP PCR CleanUp from the -20 °C freezer.
2. Keep PureIT ExoZAP PCR CleanUp on ice at all times.
3. Add 2 µl PureIT ExoZAP PCR CleanUp to 5 µl of PCR product.
4. Mix well and spin down.
5. Incubate the reaction at 37 °C for 2-5 minutes to degrade remaining primers and to inactivate excess nucleotides by dephosphorylation.
6. Incubate at 80 °C for 3-10 minutes to completely inactivate PureIT ExoZAP PCR CleanUp.
7. The cleaned up PCR product can now be used for downstream applications such as DNA sequencing, primer extension experiments or SNP analysis.
8. After treatment the PCR products can be stored at -20 °C

The protocol is scalable for larger volumes of PCR reaction mix. Simply add the according volume of PureIT ExoZAP PCR CleanUp.

Improved Sanger sequencing results after treatment with PureIT ExoZAP PCR CleanUp

For optimal performance of Sanger sequencing a PCR clean-up is required in order to remove excess primers and dNTPs. Excess primers and unincorporated dNTPs in a PCR reaction might interfere with downstream applications such as Sanger sequencing. Here, PCR clean-up is performed enzymatically using PureIT ExoZAP PCR CleanUp and a PCR clean-up reagent from a competing brand. Both PCR clean-up reagents mediate enzymatic PCR clean-up with a one-step 5 min protocol using HL-ExoI for degradation of residuals primers and recombinant SAP for dephosphorylation of dNTPs.

PCR product of 866 bp was amplified using TEMPase DNA Polymerase in 10x Ammonium Buffer according to protocol recommendations. The 866 bp PCR product was hereafter spiked with dNTPs and primers and used for evaluating the functional performance of PureIT ExoZAP PCR CleanUp and the competing PCR clean-up reagent. The spiked 866 bp PCR product was treated with and without the two PCR clean-

up reagents. Sanger sequencing was used to analyze the treated and untreated PCR product. By graphically plotting the number of Quality Values against base calls the functional equivalency of the two PCR clean-up kits could be compared. All samples were treated in accordance with supplier's manuals and analyzed in triplicates. Figure 2.

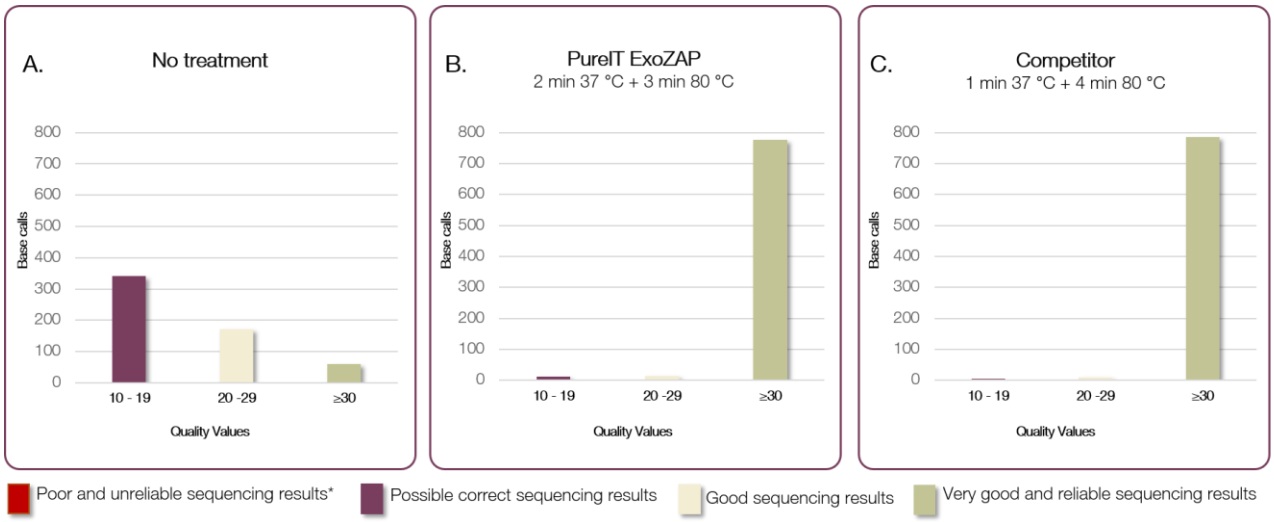

Figure 2. *Presentation of Sanger sequencing results by plotting the number of base calls with certain quality values (Phred score). A. No treatment. B. Treatment with PureIT ExoZAP PCR CleanUp (2 + 3 min). C. Treatment using competitor product (1 + 4 min). * Data with Quality Values <10 is not included in figure 2.*

Electropherograms of the 866 bp PCR product treated with and without PureIT ExoZAP PCR CleanUp is shown in figure 3.

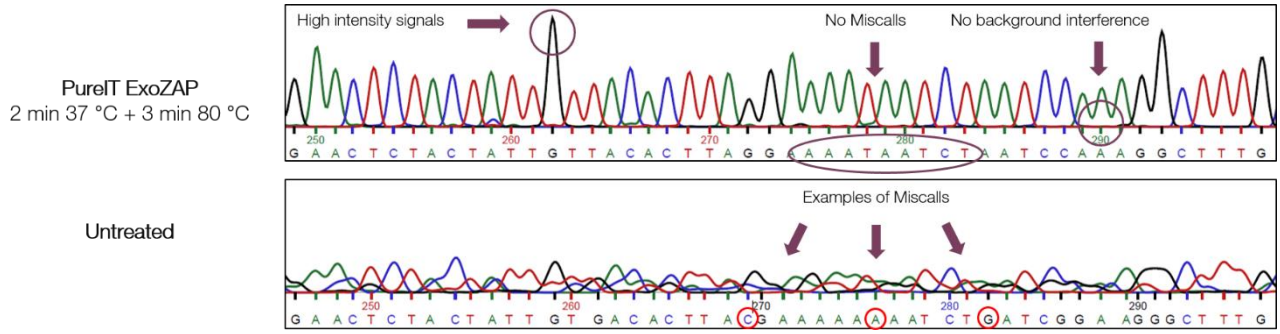

Figure 3. *Sanger sequencing results of the 866 bp PCR product. The depicted electropherograms are either after treatment with PureIT ExoZAP PCR CleanUp (top) or untreated (bottom). Sequence shown is approximately the region from 250 - 300 bases.*

Conclusion

Treatment of spiked PCR products with PureIT ExoZAP PCR CleanUp significantly improves the quality of Sanger sequencing as observed in the electropherograms. Figure 3. The benefit of using PureIT ExoZAP PCR CleanUp for PCR clean-up is very clear, as it results in enhanced signal intensity and eliminates background interference and base miscalls.

PureIT ExoZAP PCR CleanUp treatment was compared to a market leading PCR clean-up reagent by plotting Quality Values against base calls. Figure 2. Under the conditions tested here PureIT ExoZAP PCR CleanUp showed PCR clean-up results equivalent to the competing PCR clean-up reagent. This study supports equivalent functional performance by PureIT ExoZAP PCR CleanUp and the evaluated competitor in the presence of excess levels of primers and dNTPs.

Ordering information

Cat. No.	Reactions	PureIT ExoZAP PCR CleanUp
A620601	100	1 x 0.2 ml
A620603	500	1 x 1 ml
A620606	2500	5 x 1 ml
A620607	5000	10 x 1 ml

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.