ExoSAP-IT® PCR Cleanup Reagents

ExoSAP-IT PCR Product Cleanup (original formulation) [PN 78200/01/02/05/50]
ExoSAP-IT Express PCR Product Cleanup [PN 75001/02]
HT ExoSAP-IT Fast High-Throughput PCR Product Cleanup [PN 78595]

General

1. **What is ExoSAP-IT PCR Product Cleanup reagent?**
   ExoSAP-IT PCR Product Cleanup is a single tube reagent for enzymatic cleanup of PCR products. It contains Exonuclease I for degradation of unused single-stranded primers and Shrimp Alkaline Phosphatase (SAP) for dephosphorylation of unused nucleotides. Both enzymes are irreversibly inactivated in the ExoSAP-IT protocol so that the cleaned PCR product can be used directly in downstream applications.

2. **What are the differences between versions of ExoSAP-IT reagent?**
   All versions of ExoSAP-IT reagent have a similar function to cleanup PCR products, but differ per the table below:

<table>
<thead>
<tr>
<th></th>
<th>ExoSAP-IT® Express</th>
<th>ExoSAP-IT reagent</th>
<th>HT ExoSAP-IT Fast High-Throughput</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protocol time</strong></td>
<td>5 minutes</td>
<td>30 minutes</td>
<td>14 minutes</td>
</tr>
<tr>
<td><strong>Format</strong></td>
<td>Single tube*</td>
<td>Single tube</td>
<td>Single tube</td>
</tr>
<tr>
<td></td>
<td>8-tube strips</td>
<td></td>
<td>8-tube strips</td>
</tr>
<tr>
<td></td>
<td>96-well plate*</td>
<td></td>
<td>96-well plate</td>
</tr>
<tr>
<td><strong>Throughput level</strong></td>
<td>Low to high;</td>
<td>Low to mid;</td>
<td>High;</td>
</tr>
<tr>
<td></td>
<td>Recommended for processing any sample size</td>
<td>Recommended for processing 1-96 samples at a time</td>
<td>Recommended for processing ≥ 96 samples at a time</td>
</tr>
<tr>
<td><strong>Platform</strong></td>
<td>Single- or multi-channel pipette, automated liquid handling platforms</td>
<td>Single-channel pipette</td>
<td>Automated liquid handling platforms</td>
</tr>
<tr>
<td><strong>Freezes at -20°C</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>-20°C for up to 2 years</td>
<td>-20°C for up to 2 years</td>
<td>-20°C for up to 2 years; Once thawed, stable at 4°C for 1 month and RT for 2 days</td>
</tr>
</tbody>
</table>

*Optional tracking dye available (PN 75002).
3. How should ExoSAP-IT reagent be stored?
ExoSAP-IT reagent should be stored at -20°C in a non-frost-free freezer.

4. What are the differences in the protocols between the different versions of ExoSAP-IT reagent?
All versions of ExoSAP-IT reagent follow a simple one-pipette step protocol where 2 µl ExoSAP-IT reagent is added to 5 µl PCR product followed by incubation at 37°C for enzyme activation, then 80°C for inactivation. The incubation times differ as follows:
- ExoSAP-IT reagent (original formulation): 15 minutes at 37°C; 15 minutes at 80°C
- HT ExoSAP-IT Fast reagent: 7 minutes at 37°C; 7 minutes at 80°C
- ExoSAP-IT Express reagent: 4 minutes at 37°C; 1 minute at 80°C

5. Can PCR products be stored after cleanup with ExoSAP-IT reagent?
Yes. Treated PCR products may be stored at -20°C until required.

6. What is the percent recovery of PCR products treated with ExoSAP-IT reagent?
100% recovery. Unlike other techniques such as spin columns, magnetic beads, and precipitation, no yield is lost during ExoSAP-IT treatment and the cleaned PCR products can be used directly in downstream applications.

7. Is there an amplicon length requirement for ExoSAP-IT reagent?
No. ExoSAP-IT PCR product cleanup results in 100% yield, regardless of amplicon length.

8. What are the key applications for ExoSAP-IT reagent?
ExoSAP-IT reagent is routinely used for cleaning up PCR products for Sanger sequencing. It is also used for PCR cleanup in workflows that involve downstream primer extension, subsequent rounds of PCR, and denaturation followed by probe hybridization. These applications include SNP analysis, HLA typing, amplicon-based NGS, bead-based arrays, and microarrays.

9. How does ExoSAP-IT PCR cleanup improve sequencing results?
ExoSAP-IT treatment prior to sequencing eliminates miscalls, improves quality scores, increases continuous read length (CRL), and decreases background interference. Altogether, this results in superior sequencing results with greater confidence, consistency, and accuracy.

10. Can ExoSAP-IT-treated PCR products be used in cloning applications?
ExoSAP-IT-treated PCR products can be used in TA cloning, as Exonuclease I in ExoSAP-IT reagent will not degrade the single A-overhang required for TA cloning. For additional information on using ExoSAP-IT reagent in cloning applications, please contact USB Technical Support at USBtechsupport@affymetrix.com.

11. Can ExoSAP-IT-treated PCR products be used in 5’-end labeling reactions to make probes?
Yes, but with limited efficiency, as ExoSAP-IT treatment generates mono and dinucleotides as well as inorganic phosphate and pyrophosphate, all of which can decrease the efficiency of the downstream kinase reaction.

12. Can ExoSAP-IT-treated PCR products be used for in vitro transcription?
Yes. ExoSAP-IT treatment can remove the primers containing the RNA polymerase promoter that will be used in a subsequent transcription reaction, which ensures that transcription will occur from desired PCR products and not from excess primers.
13. Can ExoSAP-IT reagent be used to cleanup PCR products from multiplex reactions?
   Yes. In cases where significant primers/dNTPs are leftover, ExoSAP-IT cleanup can be optimized by adding an additional 15 minutes to the 37°C incubation step.

14. Can ExoSAP-IT reagent be used to cleanup PCR products amplified using High Fidelity (HiFi) DNA polymerases?
   The proofreading function of HiFi DNA polymerases can cause partial degradation of PCR products during extended incubation at 80°C. ExoSAP-IT Express reagent, requiring only 1 minute at 80°C for inactivation, is recommended for HiFi PCR cleanup. For additional information regarding HiFi PCR cleanup with ExoSAP-IT reagent, please contact USB Technical Support at USBtechsupport@affymetrix.com.

15. Which version of ExoSAP-IT reagent should be used when cleaning many PCR samples at once?
   HT ExoSAP-IT Fast reagent and ExoSAP-IT Express reagent are offered in 8-tube strip and 96-well formats, which are conveniently configured for use with multi-channel pipettes and automated liquid handlers. HT ExoSAP-IT Fast reagent is formulated at a lower viscosity that is more conducive for automation and high-throughput labs where the reagent is consumed in a small number of runs. ExoSAP-IT Express reagent is formulated at a higher viscosity to prevent freezing, which is advantageous when re-using the reagent over multiple runs.

ExoSAP-IT Express PCR Product Cleanup [PN 75001/02]

16. How does ExoSAP-IT Express reagent inactivate so quickly at 80°C?
   ExoSAP-IT Express reagent contains a novel Exonuclease I enzyme that has been engineered by USB scientists to enable rapid, irreversible inactivation at 80°C in only 1 minute.

17. What is the tracking dye in ExoSAP-IT Express with Tracking Dye [PN 75002]?
   It is an inert blue color dye for easy tracking of ExoSAP-IT Express reagent as it is pipetted into wells for PCR cleanup.

18. What are the benefits of having a tracking dye?
   It enables visual confirmation of aspiration, dispensing, and reaction mix homogeneity. This is especially useful when using a multi-channel pipette; never lose track of your place on a plate again.

19. Does the tracking dye affect the performance or stability of ExoSAP-IT Express reagent?
   No.

20. Does the tracking dye affect downstream processing such as subsequent PCR and Sanger sequencing with Big Dye Terminators?
   No. The dye has no inhibitory effect on downstream applications.

21. Is the tracking dye removed during Big Dye Terminator cleanup in Sanger sequencing workflows?
   Yes. Since common techniques for purification after the Big Dye Termination reaction involve buffer exchange, the dye is removed during this step.

HT ExoSAP-IT Fast High-Throughput PCR Product Cleanup [PN 78595]

22. Why does the HT ExoSAP-IT Fast reagent freeze at -20°C, but not the ExoSAP-IT original and Express reagents?
   HT ExoSAP-IT Fast reagent is formulated at a lower viscosity that is more conducive for automated liquid handling. Due to the lower viscosity formulation, HT ExoSAP-IT Fast reagent will freeze at -20°C.

23. Should multiple freeze/thaw cycling be prevented when using HT ExoSAP-IT Fast reagent?
   Yes. Due to the lower viscosity formulation, HT ExoSAP-IT Fast reagent will freeze at -20°C. The reagent should be thawed on ice and total freeze/thaw cycles limited to less than 4. The reagent can be stored at 4°C once thawed for up to 1 month.

24. What automated liquid handling instruments are compatible with HT ExoSAP-IT Fast 96-well plate format?
   HT ExoSAP-IT Fast reagent is formulated at a lower viscosity so that it is within the typical viscosity tolerance of most liquid handlers. Some popular instruments include Thermo Fisher Scientific Versette™, Perkin Elmer Janus®, Beckman Coulter® Biomek®, Gilson, Inc. PIPETMAX®, Hamilton Microlab® NIMBUS®, Tecan Freedom EVO®, Agilent Bravo, Qiagen® QIAgility®, Aurora Biomed VERSA™, and Eppendorf epMotion®.
ExoSAP-IT reagents in Next Generation Sequencing

25. Can ExoSAP-IT reagent be used for cleanup steps in Next Generation Sequencing (NGS) workflows?

Yes, ExoSAP-IT reagent can be used to remove primers and nucleotides between amplification steps in NGS workflows that use a PCR-based strategy to amplify targets and add adapters/indices. ExoSAP-IT reagent can also be used for post-library amplification cleanup, but additional processing steps might be required when adapter dimers are prevalent. For additional information on using ExoSAP-IT reagent in NGS, please contact USB Technical Support at USBtechsupport@affymetrix.com.

26. What are the primary uses for ExoSAP-IT reagent in NGS workflows?

- **Amplicon-based library prep**—ExoSAP-IT reagent is used to remove primers and nucleotides between amplification steps in targeted amplicon sequencing workflows that use tagged primers to add adapters and indices.
- **Enzymatic shearing of PCR products**—ExoSAP-IT reagent is used to remove ssDNA that can interfere with the enzymatic shearing step by Ion Shear™ Plus Reagent within the Ion Xpress™ Plus Fragment Library Kit.

27. What are the advantages of using ExoSAP-IT reagent to replace bead- or column-based cleanup steps in NGS workflows?

- **100% recovery**—Where bead- and column-based techniques can result in low yields and favor products of certain length, ExoSAP-IT PCR cleanup results in 100% recovery regardless of amplicon length.
- **Faster protocol**—Where bead and column-based techniques involve multiple steps and are challenged by throughput, ExoSAP-IT PCR cleanup is a fast (as little as 5 minutes), one-step protocol with unlimited scalability.
- **Enables multiple in-line processing steps**—ExoSAP-IT reagent is compatible with most downstream processing steps so workflows can proceed through multiple steps within the same reaction tube.

28. Can ExoSAP-IT reagent be used to remove primers and nucleotides from reverse transcribed cDNA in RNA-Seq workflows?

Yes, as long as the cDNA is in a duplex with RNA. RNase H- (minus) reverse transcriptases, such as SuperScript™ II/III and VeriScript™ Reverse Transcriptase [PN 78070], are necessary when making cDNA to be cleaned with ExoSAP-IT reagent.

29. How can PCR products be quantified after cleaning with ExoSAP-IT reagent?

Quant-iT™ PicoGreen® dsDNA Assay Kit is recommended for quantifying PCR products that have been cleaned with ExoSAP-IT reagent. This technique enables equal molar pooling of separate PCR reactions for subsequent amplification.

30. Can ExoSAP-IT reagent remove adapter dimers?

No. ExoSAP-IT reagent does not digest dsDNA, which is why it leaves PCR products and primer dimers undigested. To eliminate primer dimer formation, it is recommended to check for optimal primer design and use PCR polymerases with hot start technology or setup reactions on ice. Additionally, HotStart-IT® Binding Protein [PN 71194] can be added to PCR reactions to prevent primer dimers and increase yield and specificity.

31. What NGS library preparation kits are compatible with ExoSAP-IT reagent?

ExoSAP-IT reagent has been used to cleanup PCR products prior to processing with Thermo Fisher Scientific Ion Xpress Plus Fragment Library Kit and Illumina® Nextera® XT DNA Sample Preparation Kit.