PCR cleanup

HT ExoSAP-IT High-Throughput PCR Product Cleanup

Applied Biosystems™ ExoSAP-IT™ cleanup reagent revolutionized PCR cleanup for general sequencing applications. Applied Biosystems™ HT ExoSAP-IT™ High-Throughput PCR Product Cleanup reagent is an alternative formulation specifically designed for the unique requirements of automated high-throughput platforms. Like ExoSAP-IT reagent, HT ExoSAP-IT reagent is a mixture of exonuclease I and shrimp alkaline phosphatase (SAP) that enzymatically removes excess primers and dNTPs in one step after PCR. HT ExoSAP-IT reagent is less viscous than ExoSAP-IT reagent, so it facilitates robotic pipetting with the same convenience and stability of the original product. HT ExoSAP-IT reagent can be used for PCR cleanup in single tubes, 96-well plates, and 384-well plates.

Features and benefits

- **One-tube PCR cleanup**—add HT ExoSAP-IT reagent directly to PCR products
- **100% sample recovery**—no loss of PCR product regardless of fragment size
- **Simple one-step cleanup**—does not require multiple steps, so PCR cleanup with HT ExoSAP-IT reagent is faster than cleanup with beads or columns
- **High-throughput processing**—low-viscosity formulation enables robotic pipetting
- **Removes excess primers and dNTPs**—primers and dNTPs will not interfere with downstream applications such as sequencing, SNP analysis, single-base extension, or fragment analysis
- **Scalable**—to treat PCR product volumes >5 µL, simply increase the amount of HT ExoSAP-IT reagent proportionally
- **Convenient packaging**—available in single tubes, 8-tube strips, and 12-strip format in low-skirted 96-well plates.
- **Stable**—HT ExoSAP-IT reagent remains fully functional and stable for one week at 4°C or 8 hours at 25°C

High quality, accurate results

HT ExoSAP-IT High-Throughput PCR Cleanup reagent is designed to help ensure accuracy and consistency for high-throughput applications. As a test, a 1,007 bp sequence in the human *JAG1* gene was amplified from 15 ng of genomic DNA in a 100 µL reaction. A 5 µL aliquot of the PCR reaction mixture was treated with HT ExoSAP-IT reagent, and another 40 µL was processed with purification beads according to the product instructions. The fragment was then sequenced.

Robust and accurate data were obtained after cleanup with HT ExoSAP-IT reagent. Reads for PCR products treated with HT ExoSAP-IT reagent were 51 bases longer on average than reads for PCR products treated with beads. PCR products treated with HT ExoSAP-IT reagent also had higher Phred 20 quality scores than PCR products treated with beads (Table 1). Sequencing revealed the miscalling of 25 DNA bases after bead-based cleanup, which was likely due to sample loss. Recovery was 100% after treatment with HT ExoSAP-IT reagent, and there were no miscalls (Figure 1).

100% recovery over a range of fragment sizes after cleanup with HT ExoSAP-IT reagent

HT ExoSAP-IT reagent enabled 100% recovery and effectively cleaned amplicons ranging from 86 bp to 1,007 bp in length (Table 2). The beads were ineffective for recovering small amplicons, as determined by image analysis (Figure 2) and an Invitrogen™ Quant-iT™ PicoGreen™ assay.

Table 1. PCR cleanup with HT ExoSAP-IT reagent improves sequencing accuracy.

	HT ExoSAP-IT reagent	Purification beads
Number of bases read (average of 16 samples)	901	850
Total miscalls	0	25
Pass rate (%)*	100%	99.7%
CRL20**	822 ± 9	776 ± 83

* Pass rate is the average percentage of bases between base pair number 100 and 300 with a quality value (QV) ≥20.
** Contiguous read length 20 (CRL20): the maximum number of contiguous bases with an average QV ≥20, calculated over a 21 bp sliding window.

Table 2. DNA recovery after PCR cleanup.

PCR product length (bp)	DNA recovery after cleanup with beads	DNA recovery after cleanup with HT ExoSAP-IT reagent
86	10%	100%
103	12%	100%
545	63%	100%
1,007	88%	100%

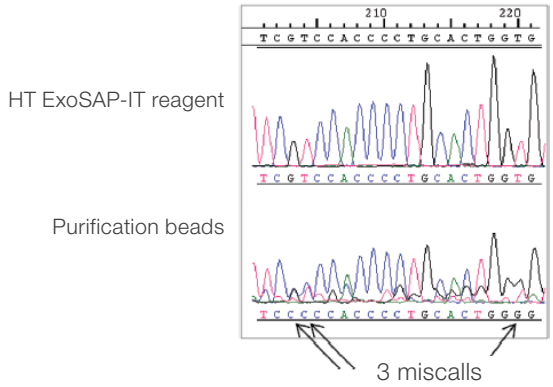

Figure 1. Sequencing of a 1,007 bp PCR product. A 1,007 bp fragment was amplified and then either treated with HT ExoSAP-IT reagent (top) or purified using beads (bottom), then sequenced. Chromatography revealed no miscalls after cleanup with HT ExoSAP-IT reagent. Miscalls were observed at positions 203, 204, and 220 after the bead-based cleanup.

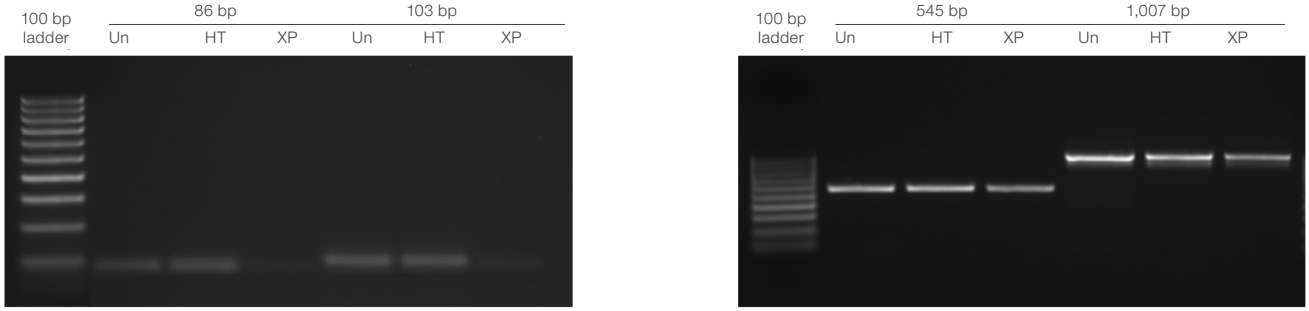

Figure 2. Higher recoveries of PCR products after treatment with HT ExoSAP-IT reagent. Equal volumes of PCR products were visualized on an agarose gel with ethidium bromide, and band volumes were determined by image analysis. After treatment with HT ExoSAP-IT reagent, 100% of the 86 bp and 103 bp PCR products were recovered. Only 8% of the 86 bp product and 14% of the 103 bp product were recovered after bead-based cleanup. Un: untreated; HT: treated with HT ExoSAP-IT reagent; XP: treated with beads.

PCR products treated with HT ExoSAP-IT reagent are stable

HT ExoSAP-IT reagent is rigorously tested and subjected to strict quality control measures to help ensure the integrity of treated PCR products. No degradation of the 1,007 bp PCR product treated with HT ExoSAP-IT reagent was observed after one week of storage at 25°C (Figure 3).

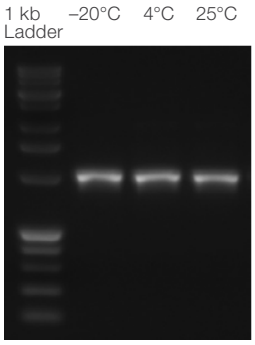

Figure 3. High stability of PCR products treated with HT ExoSAP-IT reagent. A 1,007 bp DNA fragment amplified from human genomic DNA was treated with HT ExoSAP-IT reagent and stored for 7 days at –20ºC, 4ºC, or 25ºC. Samples were visualized on a 1.5% agarose TAE gel. Ladder: Invitrogen™ 1 Kb Plus DNA Ladder.

Ordering information

Description	Cat. No.
HT ExoSAP-IT High-Throughput PCR Product Cleanup	
480 reactions	783951EA
5,760 reactions	783951PK
23,040 reactions	783954PK
1,000 reactions (2 mL)	783951000RX
5,000 reactions (10 mL)	783955000RX

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