

Data Sheet

USB® HT ExoSAP-IT® High-Throughput PCR Cleanup

USB ExoSAP-IT cleanup reagent, which revolutionized PCR cleanup for general sequencing, extends the revolution to high-throughput sequencing with HT ExoSAP-IT reagent. HT ExoSAP-IT reagent is an alternative formulation of ExoSAP-IT reagent specifically designed for the unique requirements of high-throughput, automated platforms. HT ExoSAP-IT reagent has a decreased viscosity for robotic pipetting yet maintains the same convenience and stability that you have come to expect from the ExoSAP-IT products. Like ExoSAP-IT, HT ExoSAP-IT reagent is a mixture of Exonuclease I and Shrimp Alkaline Phosphatase, Recombinant for the enzymatic removal of excess primers and dNTPs following a PCR reaction. Also, like ExoSAP-IT reagent, HT ExoSAP-IT reagent allows PCR cleanup with a single pipetting step. HT ExoSAP-IT reagent is scalable for PCR cleanup in a single tube up to a 384-well plate PCR reaction.

Features and benefits

- **One-tube PCR cleanup**—Add HT ExoSAP-IT reagent directly to PCR product
- **100% sample recovery**—No loss of PCR products regardless of the fragment size
- **Simple, single-step**—Replaces the multiple steps and wasted time inherent with beads or columns (see table)
- **High-throughput processing**—Low viscosity formulation allows robotic pipetting
- **Removes excess primers and dNTPs**—Does not interfere with downstream applications
 - sequencing
 - SNP analysis
 - single base extension
 - fragment analysis
- **Scalable**—Treat reaction volumes from 5 µl to 5 L
- **Convenient packaging**—Available in 8-tube strips and 96-well formats
- **Stable at 25°C for 8 hours**—Retains full functional activity and is stable for one week at 4°C

High quality – accurate results

HT ExoSAP-IT High-Throughput PCR Cleanup reagent is designed to provide accurate and consistent results in high-throughput applications. Identical PCR products were purified with HT ExoSAP-IT reagent and with AMPure® XP beads, and the treated PCR products were then sequenced. The results highlight the robust and accurate data obtainable with HT ExoSAP-IT reagent. A 1,007 bp PCR fragment from human jagged1 was amplified from 15 ng of human genomic DNA in a 100 µl reaction using HotStart-IT® Fidelity™ PCR Master Mix

(PN 71156). A sample of 5 µl of the PCR reaction was treated with HT ExoSAP-IT reagent and 40 µl of PCR reaction was processed with Agencourt® AMPure XP beads, both according to the manufacturer's directions.

Read lengths from PCR products treated with HT ExoSAP-IT reagent were on average 51 bases longer than those from PCR products purified with AMPure XP beads. In addition, the HT ExoSAP-IT-treated PCR products had higher Phred 20 scores than the AMPure XP purified PCR products (Table 1). Thus, HT ExoSAP-IT reagent allowed longer read lengths and greater confidence in the accuracy of those reads than did AMPure XP beads. Sequencing also revealed that the bead-purified DNA had base miscalls, which is likely an effect of sample loss. HT ExoSAP-IT-treated samples showed no miscalls and had 100% recovery (Fig. 1).

Table 1. HT ExoSAP-IT reagent gives better sequencing results.

	HT ExoSAP-IT reagent	AMPure XP beads
Number bases read (Average of 16 samples)	901	850
Total miscalls	0	25
Pass rate (%)*	100%	99.7%
Phred 20**	822 ± 9	776 ± 83

* Pass rate – Average Phred value greater than 20 bases between 100 bp and 300 bp.

** Phred 20 values over the entire length of sequence (no trimming –970 bp)

Fig. 1. Sequencing of a 1,007 bp treated PCR product

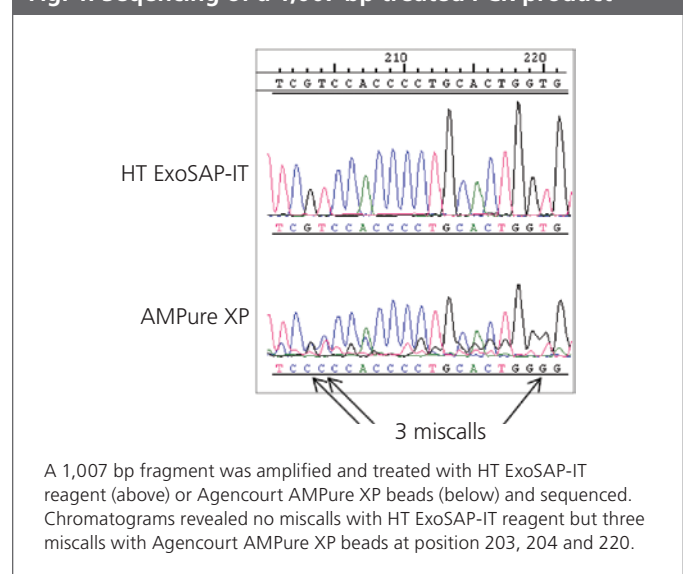
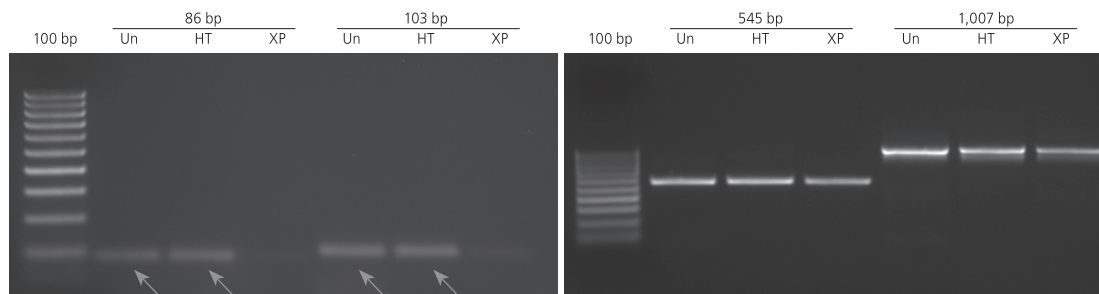


Fig. 2. Greater recovery of PCR product with HT ExoSAP-IT reagent



Equivalent volumes of PCR product were visualized on an ethidium bromide agarose gel and the band volume determined using image analysis. HT-ExoSAP-IT reagent allowed recovery of 100% of the 86 and 103 bp PCR products but AMPure XP beads allowed only 8 and 14% recovery, respectively. Un – Untreated, HT – HT-ExoSAP-IT treated, XP – AMPure XP purified, 100 bp – Affymetrix 100 bp ladder, bands from 100 to 1,000 bp by 100 bp intervals (PN 76712).

100% recovery from a range of fragment sizes with HT ExoSAP-IT reagent

HT ExoSAP-IT reagent ensures 100% recovery and provides effective cleanup of all amplicon sizes. In contrast, AMPure XP beads were ineffective at purifying small amplicons whether determined by image analysis (Fig. 2) or by PicoGreen assay (Table 2).

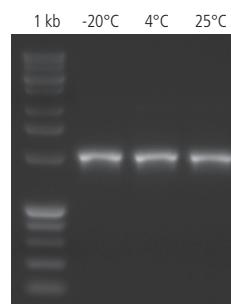
Table 2. HT ExoSAP-IT reagent gives 100% recovery.

PCR size	DNA recovery after purification with Agencourt AMPure XP beads	DNA recovery after purification with HT-ExoSAP-IT reagent
86 bp	10%	100%
103 bp	12%	100%
545 bp	63%	100%
1,007 bp	88%	100%

HT ExoSAP-IT-treated PCR product is stable

HT ExoSAP-IT reagent is rigorously tested and subject to strict quality control. This helps to insure the integrity of the treated PCR product. Fig. 3 demonstrates that no product degradation occurred after storage of HT ExoSAP-IT-treated PCR product for one week at 25°C.

Fig. 3. High stability of HT ExoSAP-IT-treated products



A 1,007 bp DNA fragment amplified from genomic DNA with HotStart FidelityTaq PCR Master Mix (PN 71156) was treated with **HT ExoSAP-IT reagent** and stored for seven days at: -20°C (lane 1), 4°C (lane 2), or 25°C (lane 3). Samples were visualized on 1.5% agarose/TAE/ethidium bromide gel. Lane MW: DNA Ladder, 1 kb Plus (PN 76714).

HT ExoSAP-IT reagent

Product Code	Pack Size
78395	1 each – 480 reactions x 8-tube strip
	1 pack – 5,760 reactions x 1 plate (12 x 8-tube strips)
	4 pack – 23,040 reactions x 4 plates
	1,000 reactions – 2 ml
	5,000 reactions – 10 ml

For research use only. Not for use in diagnostic procedures.

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Affymetrix, Inc.

USB® Products

26111 Miles Road
Cleveland Ohio 44128
Tel: 888-362-2447 | 216-765-5000
Fax: 800-535-0898 | 216-464-5075
USBcustomerserv@affymetrix.com
usb.affymetrix.com

Affymetrix UK Ltd.

USB® Products

Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH, United Kingdom
Tel: +44 (0)1628 55 2600
Fax: +44 (0)1628 55 2675
USBcustomerserveurope@affymetrix.com

Affymetrix Pte Ltd

USB® Products

7 Gul Circle, #2M-01
Keppel Logistics Building
Singapore 629563
Tel: +65 63957301
Fax: +65 63957300
USBcustomerserv@affymetrix.com