ExoSAP-IT PCR Clean-up Kit

Description

ExoSAP-IT™ is designed for fast and efficient purification of Polymerase Chain Reaction (PCR) products for downstream applications such as sequencing, genotyping (SNP analysis), cloning, *in vitro* transcription, etc.

ExoSAP-IT consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

ExoSAP-IT offers:

- A 30–min PCR clean-up protocol in a convenient one-tube, one-step format
- 100% recovery for both small (< 100 bp) and long (> 20 kb) PCR products
- Less hands-on time compared to other methods
- A scalable and easy to automate procedure for handling large volumes

Quick: 30 min clean-up protocol

The protocol for ExoSAP-IT consists of a single pipetting step (enzyme mixture addition), a 15-min incubation at 37 °C followed by enzyme inactivation at 80 °C for a further 15 min. Thus the clean-up procedure is completed within 30 min and your PCR product is ready to be used in downstream applications (Fig 1). The heat-inactivated enzymes do not interfere with downstream applications.

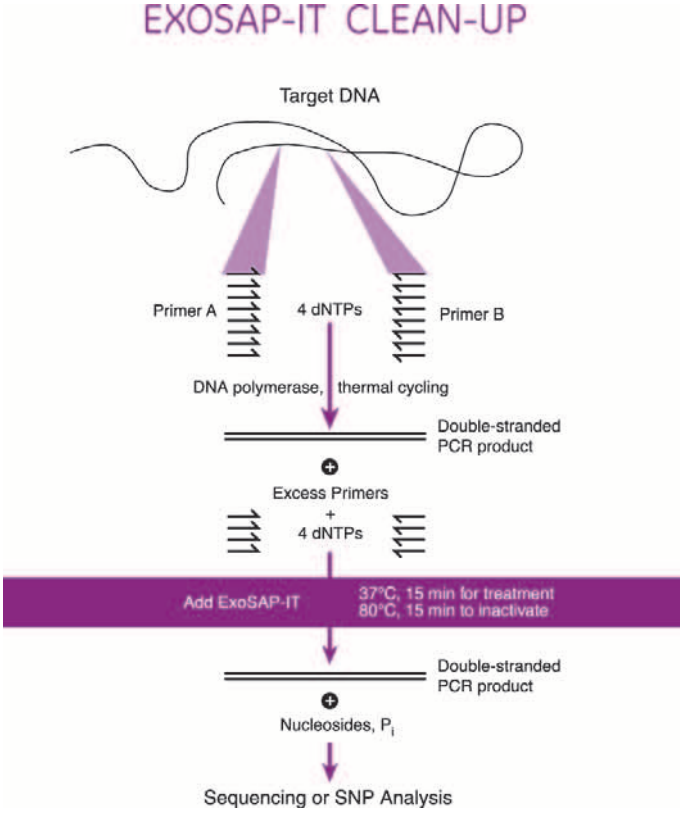

Fig 1. Schematic diagram of the ExoSAP-IT PCR clean-up method

Simple: single-step, one-tube

ExoSAP-IT eliminates the need for gel or spin column purifications, sedimentations, filtrations, beads and/or magnetic separations (1). The method requires minimum hands-on time. Both Exonuclease I and SAP are active in commonly used PCR buffers hence eliminating the need for a buffer exchange step. Exonuclease I removes residual single-stranded primers and any extraneous single-stranded DNA produced during the PCR reaction (Fig 2). SAP removes the remaining, unincorporated dNTPs from the PCR mixture.

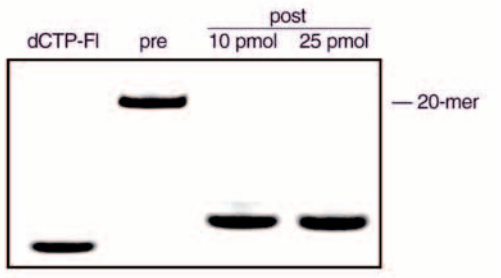

Fig 2. Perfect primer degradation. A 5' FAM-labeled 20-mer was electrophoresed on a 15% denaturating polyacrylamide gel (pre) and following (post) ExoSAP-IT treatment (0.1 pmol per lane). The oligo and subsequent digestion products were visualized on a fluorescent scanner. ExoSAP-IT is able to digest at least 25 pmol of primers (in 5µl) in 15 min at 37 °C, which is about ten times the average concentration of primers that are present in a typical PCR reaction. The digestion product is a 5' FAM-labeled dinucleotide. Fluorescein-labeled dCTP is shown as a marker.

High yield: 100% sample recovery

Unlike other protocols that use multiple steps and spin columns with recoveries in the range of 70-90%, ExoSAP-IT can be used to process PCR products ranging in size from less than 100 bp to well over 20 kb with no sample loss. The enzymatic treatment step of ExoSAP-IT ensures that all of your PCR products are retained in the reaction tube (Fig 3).

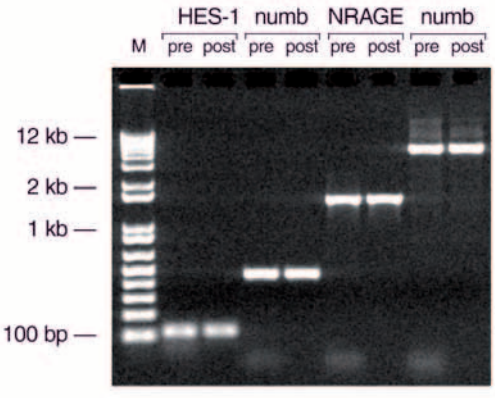

Fig 3. No loss of PCR products after ExoSAP-IT treatment. Single-copy targets were amplified from human genomic DNA. HES-1 (125 bp), numb (455 bp), NRAGE (1.55 kb) and numb (4.6 kb) were loaded on a 1.5% agarose gel before (pre) and after (post) 15 min treatment with ExoSAP-IT at 37 °C. The DNA marker is shown on lane M. A wide range of PCR product sizes can be treated with ExoSAP-IT, even a 125-bp fragment, with no sample loss.

Scalable: decreases time and expense while increasing throughput

The single pipetting step for PCR clean-up enables direct enzyme addition to the PCR reaction tube. This makes ExoSAP-IT the method of choice for processing multiple samples at once; either manually or with robotic devices.

Sequence-grade: obtain high-quality sequencing results with PCR products

Enzymatic treatment to remove excess primers and nucleotides yields templates that can be easily sequenced. Problems with leftover PCR primers leading to background bands are virtually eliminated. ExoSAP-IT can be used as an effective clean-up method prior to any fluorescent or radioactive DNA sequencing protocol (Figs 4 and 5).

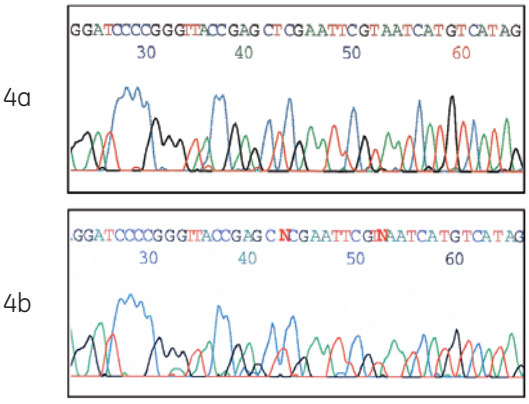

Fig 4. Fluorescent sequencing results of a 100–bp pUC18 PCR fragment. Data generated by Cleveland Genomics—a research service company. Pre-sequencing PCR clean-up performed with a) ExoSAP-IT and b) a commercial column designed for PCR clean-up. Base miscalls in b) are due to inherently low yields or short PCR products when using columns (courtesy of USB Corporation).

Fig 5. Autoradiograms of a 20.7-kb Lambda PCR fragment sequenced with Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit. Pre-sequencing PCR clean-up performed with: (a) ExoSAP-IT; (b) a commercial column designed for PCR clean-up.

Applications

ExoSAP-IT is used in an increasing range of applications including sequencing (manual and automated), genotyping (SNP analysis), cloning, *in vitro* transcription, nested PCR, etc.

Components

Exonuclease I from *E. coli* and Shrimp Alkaline Phosphatase (arctic *Pandalus borealis*) in a specially formulated buffer compatible with commonly used buffers for PCR.

Storage and Stability

Shipped on dry ice. Store at -20 °C and keep on ice during pipetting. ExoSAP-IT retains 75% of its activity after 8 h incubation at room temperature and > 50% of its activity after one week at 4 °C in its storage buffer. ExoSAP-IT can be frozen and thawed up to 4 times without significant loss of activity. Product can be subaliquoted for more convenient storage, if required.

Expiry

See details on main packaging.

References

1. Dugan, K. A. *et al.* An improved method for post-PCR purification for mtDNA sequence. analysis. *J. Forensic Sci.* **47,** 811-818 (2002)
2. Hanke, M. *et al.* Direct DNA sequencing of PCR-amplified vector inserts following enzymatic degradation of primer and dNTPs. *BioTechniques*. **17,** 858-860 (1994)
3. Mu, J. *et al.* Chromosome-wide SNPs reveal an ancient origin for Plasmodium falciparum. *Nature.* **418,** 323-326 (2002)
4. Silva, J.R., *et al.* PCR template preparation for capillary DNA sequencing. *BioTechniques*. **30,** 537-542 (2001)
5. Werle, E. *et al.* Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Res*. **22,** 4354-4355 (1994)
6. Hoppe, B. *et al.* Description of a novel HLA-B allele, B*5613, identified during HLA-typing using sequence-specific oligonucleotide hybridization and sequence-specific amplification. *Tissue Antigens*. **64,** 616-617 (2004)

Ordering Information

ExoSAP-IT One Step PCR Clean-up

Description	Code number
100 reactions	US78200
500 reactions	US78201
2000 reactions	US78202

Custom product solutions available upon request

Related products

Polymerases for PCR

Description	Code number
Taq DNA Polymerase	see catalog for full range
FIdeliTaq PCR Master Mix (2X)	E71182
FIdeliTaq PCR Master Mix Plus	E71183
RT-PCR Master Mix (2X)	E78370
PureTaq™ Ready-To-Go™ PCR Beads	see catalog for full range

GenomiPhi™ DNA Amplification Kit

Description	Code number
25 reactions	25-6600-00
100 reactions	25-6600-01
500 reactions	25-6600-02

Nucleotides

Description	Code number
dNTPs for PCR and Long PCR, Premixed	see catalog for full range

Sequenase™ Version 2.0 DNA Sequencing Kit

Description	Code number
100 templates	US70770

Thermo Sequenase™ Cycle Sequencing Kit

Description	Code number
100 reactions	US78500

Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit

Description	Code number
50 templates	US79750

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