



ExoSAP-IT®

Ecoli

kvalita za príťažlivé ceny



rýchly, bezkolónkový systém na purifikáciu PCR produktov so **100% výťažnosťou**

PCR reakcia → pridanie ExoSAP-IT → inkubácia → purifikovaný PCR produkt

- **One Tube/One Step PCR Clean-Up** — Add ExoSAP-IT directly to PCR product
- **Eliminates Spin Columns** — Decreases time and expense while increasing yield
- **Conserves PCR Samples** — 100% recovery of both short and long PCR products
- **Removes Contaminating Primers and dNTPs** — No interference in downstream applications
- **Scalable** — Economical for high throughput purification
- **Simple Processing** — Robotic-friendly; Replaces beads, filtrations, and plates

ExoSAP-IT reagent is designed for simple, quick PCR clean-up for downstream applications, such as DNA sequencing or Single Nucleotide Polymorphism (SNP) analysis. When PCR amplification is complete, any unconsumed dNTPs and primers remaining in the PCR product mixture will interfere with these methods. ExoSAP-IT removes these contaminants.

ExoSAP-IT is added directly to the PCR product and incubated at 37°C for 15 minutes. After PCR treatment, ExoSAP-IT is inactivated simply by heating to 80°C for 15 minutes.

Fig. 1. Summary of ExoSAP-IT PCR product treatment

ExoSAP-IT utilizes two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase, together in a specially formulated buffer, to remove unwanted dNTPs and primers from PCR products. Exonuclease I removes residual single-stranded primers and any extraneous single-stranded DNA produced in the PCR. Shrimp Alkaline Phosphatase removes the remaining dNTPs from the PCR mixture.

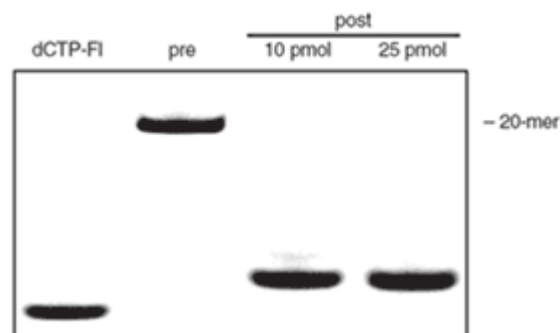
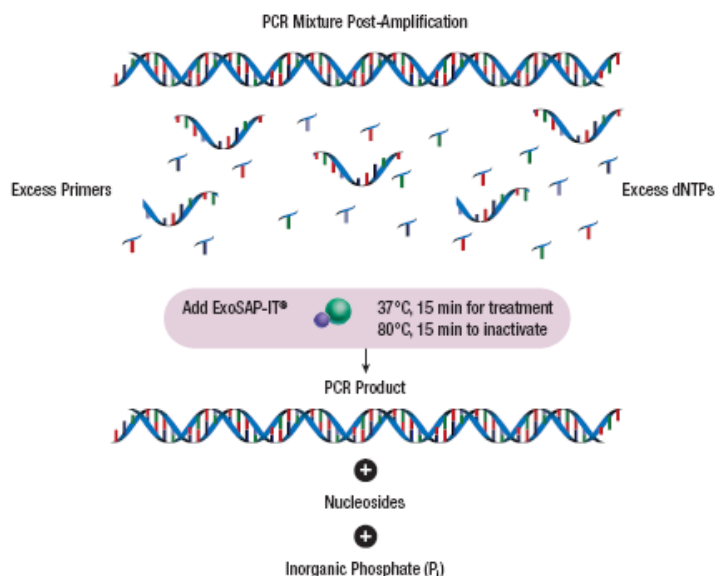


Fig. 4. ExoSAP-IT treatment of primers.

A 5' FAM-labeled 20-mer was electrophoresed on a 15% denaturing polyacrylamide gel before (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 5 µl) in 15 min at 37°C, which is about 10 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.

Rapid PCR Product Clean-Up Protocol

ExoSAP-IT requires only one pipetting step and two incubations. Just add ExoSAP-IT to the PCR product and within 30 minutes sequencing or SNP analysis can be performed.

Simple: Single-Step

The method is designed to require a minimum of 'hands-on' time. Enzymatic removal of excess primers and unincorporated nucleotides occurs in one easy step by using ExoSAP-IT reagent in a single tube or microtiter well. Only simple pipette transfers are required, therefore, many samples can be processed at once, either manually or with robotics.

No Sample Loss

Use of ExoSAP-IT eliminates all gel or column purifications, sedimentations, filtrations, beads and/or magnetic separations⁽¹⁾. There is 100% recovery of both short and long PCR products with ExoSAP-IT (fig. 2).

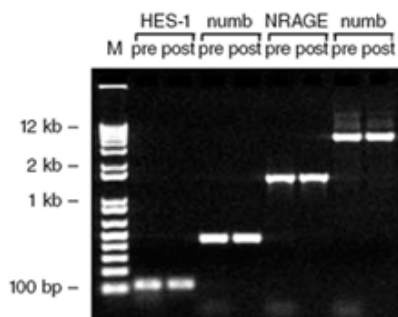


Fig. 2. ExoSAP-IT treatment of PCR products with no sample loss.

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) ExoSAP-IT treatment. M is the DNA marker lane. Note that a variety of PCR product sizes can be treated with ExoSAP-IT, even a 125 bp fragment, with no sample loss.

Achieve High Data Quality from PCR Products

ExoSAP-IT may be used as an effective clean-up method prior to fluorescent or radioactive DNA sequencing (Fig. 3), SNP analysis or any other application requiring a PCR product free of excess nucleotides and primers.

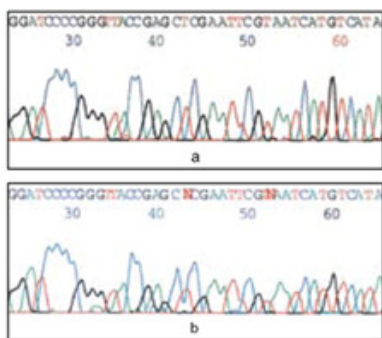


Fig. 3. Fluorescent sequencing results of a 100 bp pUC18 PCR fragment sequenced with a -20 Fwd primer using fluorescent sequencing reagents. PCR clean-up performed with: (a) ExoSAP-IT; (b) a column designed for PCR clean-up. Base miscalls in (b) are due to inherently low yields of short PCR products when using columns.