

USB® ExoSAP-IT® PCR cleanup reagent

The USB ExoSAP-IT method is a unique, patented, one-step enzymatic cleanup of PCR products that is exclusively manufactured and available through USB products from Affymetrix.

One-tube, one-step PCR cleanup

- Add ExoSAP-IT reagent directly to PCR product

Eliminates spin columns

- Decreases time and expense while increasing yield

100% sample recovery

- No loss of PCR products, regardless of the fragment size (Fig. 1)

Removes contaminating primers & dNTPs

- No interference in downstream applications

Simple processing

- Lends itself to robotics
- Replaces beads, filtrations and plates

Scaleable

- Treat reaction volumes from 5 µl to 5 L

Stable at 25°C for 8 hours

- Retains full functional activity and is stable at 4°C for one week

Sole source

- Affymetrix is the only manufacturer of USB ExoSAP-IT reagent, ensuring your lab of product consistency and integrity
- Proprietary buffer formulation for superior performance

Green option

- Generates less waste with our single-tube solution to PCR purification vs. columns

ExoSAP-IT reagent

ExoSAP-IT reagent is designed for simple, quick PCR cleanup for downstream applications, such as sequencing or 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 cleanup reagent is added directly to the PCR product and incubated at 37°C for 15 minutes. The enzymes are

active in the buffer used for PCR, so no buffer exchange is required. After PCR treatment, ExoSAP-IT reagent is inactivated simply by heating to 80°C for 15 minutes.

The ExoSAP-IT method 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 which would interfere with the subsequent reactions.

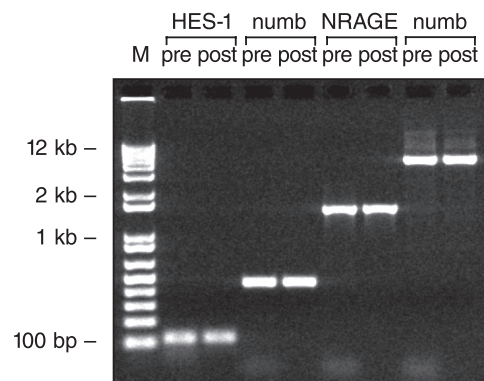
Simple: one step

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, allowing simple automated or manual processing.

100% sample recovery

Use of ExoSAP-IT reagent 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 the ExoSAP-IT method.

Fig. 1. ExoSAP-IT treatment of PCR products



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 reagent, even a 125 bp fragment, with no sample loss.

Achieve high data quality

Enzymatic treatment to remove excess primers and nucleotides yields templates which can be easily analyzed. Problems with left-over PCR primers leading to high background bands are virtually eliminated. ExoSAP-IT reagent may be used as an effective cleanup method prior to fluorescent or radioactive DNA sequencing, SNP analysis or any other application requiring a PCR product free of excess nucleotides and primers.

Storage

Store at -20°C and keep on ice while pipetting. The enzymes are heat-labile but full functional activity is retained after a week of storage at 4°C. Do not store in a frost-free freezer (the temperature rises above 0°C daily).

Composition

Exonuclease I and Shrimp Alkaline Phosphatase in specially formulated buffer.

Rapid PCR product cleanup protocol

The ExoSAP-IT method consists of one pipetting step and two incubations. Just add ExoSAP-IT reagent to the PCR product and within 30 minutes be ready to sequence or do SNP analysis.

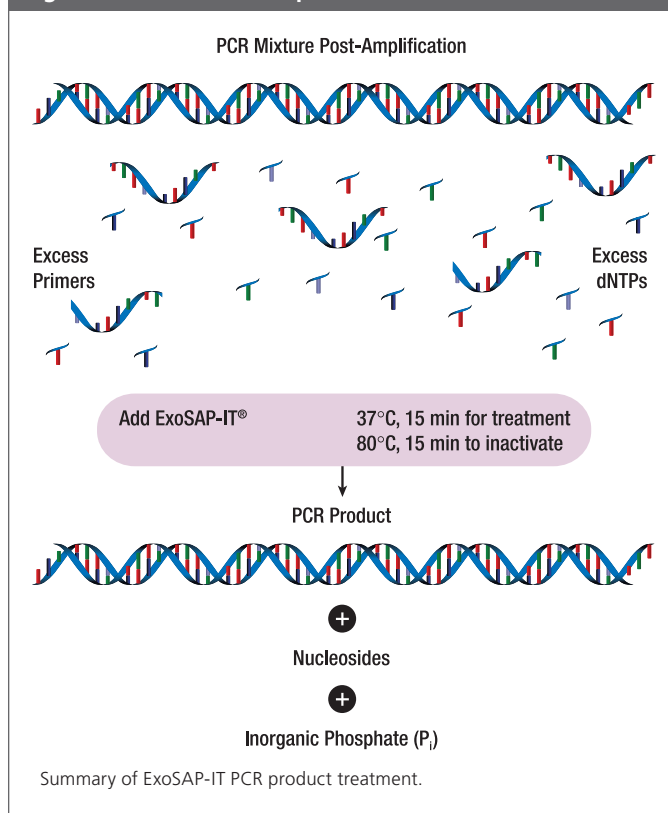
ExoSAP-IT PCR Product Cleanup

| Product code | Pack size |
|--------------|-----------------|
| 78250 | 20 reactions |
| 78200 | 100 reactions |
| 78201 | 500 reactions |
| 78202 | 2,000 reactions |
| 78205 | 5,000 reactions |

References:

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3. Mu, J., Duan, J., Makova, K., Joy, D., Huynh, C., Branch, O., Li, W. and Su, X. (2002) *Nature* **418**, 323-326.
4. Silva, Jr., W. A., Costa, M. C. R., Valente, V., De Freitas Sousa, J., Pacó-Larson, M. L., Esprefaco, E. M., Camargo, S. S., Monteiro, E., De Jesus, A., Holanda, M. A., Zago, M. A., Simpson, A. J. G. and Neto, E. D. (2001) *BioTechniques* **30**, 537- 542.
5. Werle, E., Schneider C., Renner, M., Völker, M. and Fiehn, W. (1994) *Nucleic Acids Res.* **22**, 4354-4355.

Fig. 2. ExoSAP-IT cleanup



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