

Frequently asked questions

USB® ExoSAP-IT® for PCR product cleanup [PN 78200/01/02/05/50]

What is the percent recovery of PCR product after ExoSAP-IT treatment?

ExoSAP-IT treatment results in 100% recovery of PCR products while eliminating any remaining primers and unincorporated nucleotides. This PCR purification technology is unmatched by other cleanup methods, making ExoSAP-IT a reliable and convenient method of cleaning your PCR products prior to downstream use. ExoSAP-IT treatment is carried out in only one tube, eliminating the risk of sample loss experienced when using column-based purification methods.

Can ExoSAP-IT reagent be used to cleanup PCR products prior to sequencing?

Yes. Researchers routinely utilize ExoSAP-IT to cleanup PCR products prior to DNA sequencing. Column-based purification methods can reduce the yield of PCR products which then reduces the success of downstream applications such as DNA sequencing. Treatment of PCR products with ExoSAP-IT reagent results in 100% recovery of important samples, which can then be sequenced with success.

Can ExoSAP-IT cleanup be used to degrade unincorporated primers and nucleotides from a PCR product prior to Single Nucleotide Polymorphism (SNP) analysis?

Yes. ExoSAP-IT reagent removes excess primers and nucleotides from PCR products so that SNP analysis can be carried out without complication. Affymetrix also offers researchers the SBE Cleanup Reagent [PN 78260], which is a specialized formulation of ExoSAP-IT reagent that is optimized for use with the Beckman Coulter SNPware® Core Reagent Kit [PN 101-04-300].

Can PCR products be treated with ExoSAP-IT reagent and be used directly for TA cloning?

Yes. ExoSAP-IT PCR product cleanup can be used to quickly clean PCR products prior to TA cloning without the use of purification columns or gel electrophoresis. The Exonuclease I component of ExoSAP-IT reagent does not degrade PCR product single A-overhangs required for TA cloning. ExoSAP-IT does not replace gel purification to isolate a PCR product.

What volume of ExoSAP-IT-treated PCR product should be used for TA cloning?

Typically a one to three fold molar excess of insert compared to vector is used in a ligation reaction. Appropriate volumes of ExoSAP-IT-treated insert vary depending on factors such as the amount of vector being used, as well as PCR yield. Customers have had success using 1 µl ExoSAP-IT-treated PCR product for TA cloning.

Can I use ExoSAP-IT for processing multiple PCR samples at the same time?

Yes. It makes cleaning multiple PCR samples quick, easy and reliable. ExoSAP-IT treatment of PCR products requires only a single pipetting step and two short incubations in the same tube. Entire 96-well plates can be quickly cleaned up for use in downstream applications without any sample loss.

Can the ExoSAP-IT method be used to treat PCR products before use for *in vitro* transcription?

Yes. ExoSAP-IT reagent eliminates unincorporated primers from PCR products. This is especially important when one of the primers contains the RNA polymerase promoter that will be used in a subsequent transcription reaction. ExoSAP-IT treatment ensures that transcription will occur from desired PCR products and not from excess primer.

Can ExoSAP-IT reagent be used to degrade unincorporated primers and nucleotides from a PCR product prior to a 5'-end labeling reaction to make a probe?

ExoSAP-IT reagent contains Exonuclease I and Shrimp Alkaline Phosphatase. Exonuclease I generates mono and dinucleotides, and Shrimp Alkaline Phosphatase generates inorganic phosphate and pyrophosphate. Mono and dinucleotides compete with the PCR product in kinase reactions. Inorganic phosphate and pyrophosphate may also inhibit kinase activity. Consequently, labeling efficiency may be decreased in kinase reactions following ExoSAP-IT treatment.

Can ExoSAP-IT treatment be used to digest primer dimers from PCR products?

ExoSAP-IT reagent does not digest dsDNA, which is why it leaves PCR products and primer dimers undigested. It efficiently removes ssDNA, primers and un-used nucleotides from PCR reactions. To eliminate primer dimer formation, we recommend using HotStart-IT® Binding Protein [PN 71194] with any PCR-appropriate DNA polymerase to increase yield and specificity. Additionally, optimizing primer design and PCR conditions may also be helpful.

What is the recommended storage temperature of ExoSAP-IT reagent?

ExoSAP-IT reagent should be stored at -20°C and placed on ice whenever removed from -20°C for use. ExoSAP-IT is shipped on dry ice which can result in freezing. Functional stability tests have confirmed that ExoSAP-IT can be frozen and thawed up to 8 times without significant loss of activity. We recommend avoiding repetitive freeze/thawing of ExoSAP-IT and the reagent will not freeze at -20°C.

How long does ExoSAP-IT reagent remain active at room temperature?

It retains 98% of its activity after a 2 week incubation at room temperature in its storage buffer. Activity was determined by activity assay and verified by functional assay.

How long does ExoSAP-IT reagent remain active at 4°C?

It retains 95% of its activity after two months at 4°C in its storage buffer, as determined by functional assay.

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