ExoProStar

ENZYMATIC PCR AND SEQUENCE REACTION CLEANUP

ExoProStar™ is optimized to purify polymerase chain reaction (PCR) and sequencing set up reactions quickly, efficiently and reliably.

ExoProStar contains Amersham™ Alkaline Phosphatase and Exonuclease I, formulated to work together to remove unincorporated primers and nucleotides from amplification reactions in preparation for sequencing, cloning, genotyping or further DNA modification reactions.

- Enzymes optimized to work together for high efficiency removal of unincorporated primers and nucleotides
- Enzymes provided in two separate tubes, just two simple pipetting steps are needed to prepare the reaction
- Fast 30 min protocol
- Scalable for different reaction sizes
- No loss of PCR product
- Easy to automate
- Complete heat inactivation of the enzymes within 15 min

Exonuclease I and Alkaline Phosphatase method of use is covered by US patent number 5723295 in the name of Cytiva.

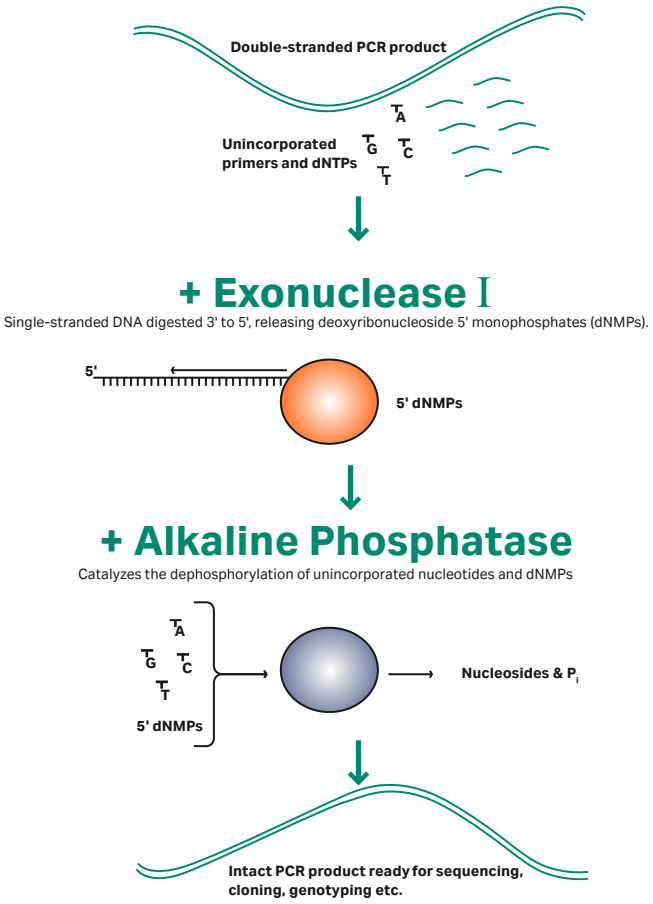

Fig 1. Schematic representation of the PCR cleanup process using ExoProStar.

Optimized for efficient primer digestion

The new Amersham Alkaline Phosphatase and Exonuclease I enzymes have been optimized for highly efficient primer digestion, helping to improve the quality of downstream analysis. In analysis of primer digestion, ExoProStar was more efficient in digesting primers than the traditional USB™ ExoSAP-IT™ product when used under the manufacturer's standard operating protocol.

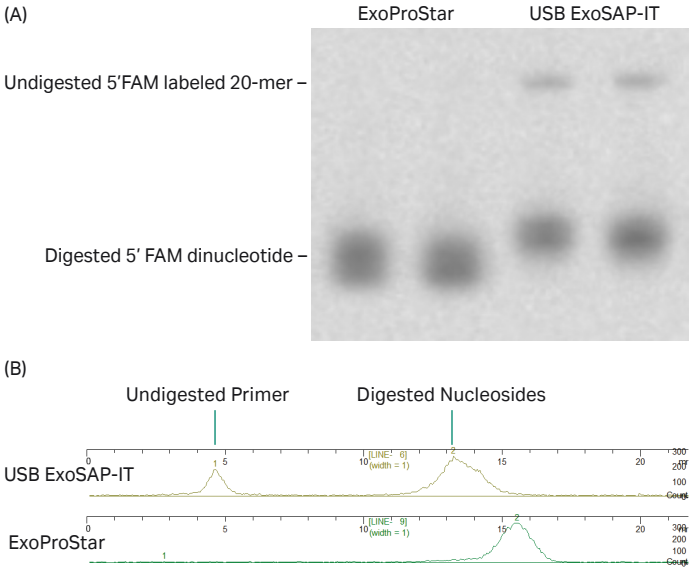

Fig 2. Panel (A), Electrophoretic analysis of the digestion of a 5'FAM labeled 20mer primer. Reactions were conducted according to manufacturer's instructions for ExoProStar and USB ExoSAP-IT using 10 pmol of primer per reaction. Panel (B), Quantitation of digestion products conducted using a Typhoon™ gel imaging system and ImageQuant™ v.5 software. No detectable primer remained in the samples using ExoProStar but undigested primer remained in samples treated with USB ExoSAP-IT.

* Data presented in Fig. 2 was obtained by scientists at Cytiva, using experimental conditions as set out in the manufacturer's operating instructions for USB ExoSAP-IT.

No loss of PCR product

The use of an enzymatic digestion approach to clean up amplification reactions reduces losses of PCR product. The process has no intermediate transfer steps, spin columns or binding matrix to retain your PCR product, and double-stranded DNA is left intact by the Exonuclease I and Alkaline Phosphatase enzymes. The size of the PCR fragment does not affect the cleanup efficiency of the reaction.

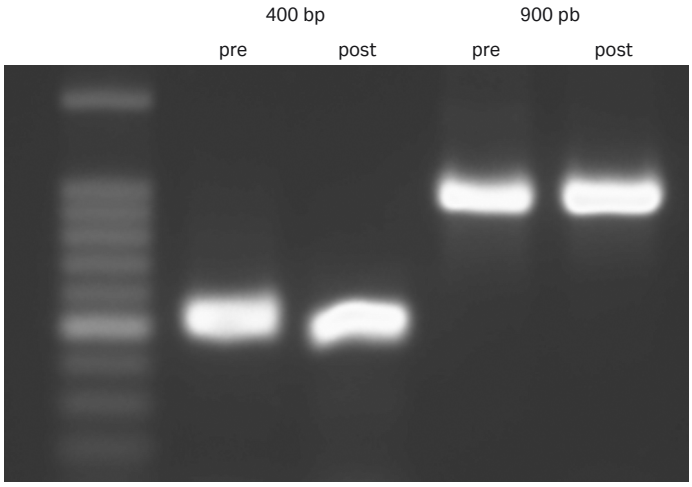

Fig 3. Agarose gel electrophoresis of different size PCR products pre- and post-digestion with ExoProStar. Samples were digested for 15 min at 37°C followed by denaturation of the ExoProStar enzymes at 80°C for 15 min as per the recommended kit protocol. No loss of PCR product was detected in any of the samples.

High quality sequencing results

Removal of unincorporated primers and nucleotides is essential to high quality DNA sequencing. Failure to fully remove these components leads to high background signals and miscalling of bases. With ExoProStar, Phred20 quality scores were routinely achieved at read lengths > 800 bp, equivalent to or better than other approaches to sample preparation.

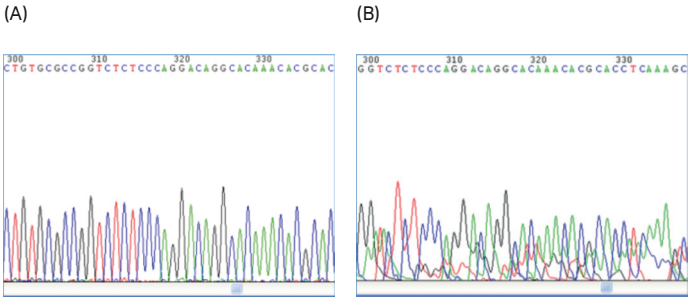

Fig 4. The importance of sample clean up before DNA sequencing is illustrated in the comparison between panel (A), showing PCR sequence quality following treatment with ExoProStar and panel (B) showing sequence quality without this treatment. Read length, base calling and sequence quality are significantly improved by the use of ExoProStar.

Heat inactivation of ExoProStar enzymes

Downstream operations can be adversely affected by the presence of active Exonuclease 1 or Alkaline Phosphatase in the PCR product following digestion. It is therefore essential that the enyzmes are effectively denatured during the post-digestion heating step.

Some alkaline phosphatase enzymes are tolerant of high temperature treatment and may retain some activity causing problems in later processes. The Amersham Alkaline Phosphatase has been optimized to be quickly denatured, reducing the risk of downstream interference.

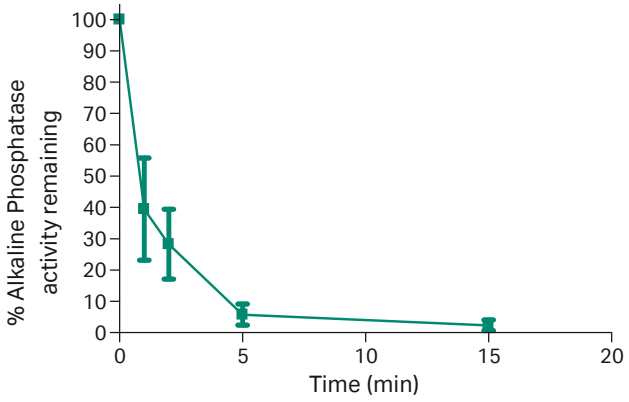

Fig 5. Temperature denaturation profile of Amersham Alkaline Phosphatase at 75°C showing rapid and complete denaturation within 15 min. The ExoProStar protocol recommends denaturation of the enzyme components at 80°C, providing greater confidence in the inactivation of both enzymes prior to further downstream processes.

Kit components and storage

The ExoProStar kit contains one tube of Exonuclease I and one tube of Amersham Alkaline Phosphatase. The kit is supplied on dry ice and should be stored at -20°C. Enzymes can be sub-aliquoted if required for storage convenience and should be maintained on ice during reaction set up.

Ordering information

ExoProStar Enzymatic PCR and Sequence Reaction Cleanup Kit

Quantity	Code number
20 reactions	US78220
100 reactions	US78210
500 reactions	US78211
2000 reactions	US78212
5000 reactions	US78225

Related products

Amplification

Product	Quantity	Code number
dNTP set (100 mM each A,C,G,T)	4 × 100 µmol	28-4065-52
Amersham Ready-To-Go™ RT-PCR Beads (0.2 ml hinged tube with cap)	96 reactions	27-9259-01
PuReTaq™ Ready-To-Go PCR Beads (0.2 ml hinged tube with cap)	96 reactions	27-9559-01
Amersham Hot Start Mix Ready-To-Go (0.2 mL tubes, 12 × 8 strip wells)	96 reactions	28-9006-53
Taq DNA Polymerase (cloned)	4 × 250 units	27-0798-05

DNA labeling

Cy™5 dUTP	250 nmol	PA55032
Cy3 dUTP	250 nmol	PA53032
Cy5 dCTP	250 nmol	PA55031
Cy3 dCTP	250 nmol	PA53031
CyDye™ Post-Labeling Reactive Dye Pack	12 × Cy3 12 × Cy5	RPN5661

DNA purification

Amersham blood genomicPrep Mini Spin Kit	50	28-9042-64
Amersham tissue and cells genomicPrep Mini Spin Kit	50	28-9042-75
Amersham bacteria genomicPrep Mini Spin Kit	50	28-9042-58

DNA cleanup

GFX™ PCR DNA and Cytival Band Purification Kit	100 purifications	28-9034-70
GFX 96 PCR Purification Kit	10 × 96 well plates	28-9034-45
Amersham MicroSpin™ S-400 HR columns	50	27-5140-01
Amersham MicroSpin S-300 HR columns	50	27-5130-01

Enzymes

Amersham Shrimp Alkaline Phosphatase	500 units	E70092Y
Exonuclease I	2500 units	E70073Z

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