ExoProStar 1-Step

ENZYMATIC PCR AND SEQUENCE REACTION CLEANUP

DOMINIQUE DUTSCHER SAS

ExoProStar™ 1-Step is optimized to purify PCR and sequencing set up reactions quickly, efficiently and reliably.

ExoProStar 1-Step contains a mix of 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 a single tube, just one simple pipetting step is required 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 both enzymes within 15 min

ExoProStar 1-Step builds on our long history and expertise in providing DNA cleanup products and expands on our original patents for enzymatic sample cleanup using Exonuclease I and Alkaline Phosphatase. With ExoProStar 1-Step we have improved on existing products to give you enhanced PCR and sequence reaction cleanup.

Rapid, simple protocol

Setting up cleanup reactions with ExoProStar 1-Step requires only a single pipetting step, thanks to the premixed enzyme formulation.

Recommended protocol

1. Remove the ExoProStar 1-Step from the freezer and keep on ice while preparing the reaction.
2. Take a 5 µl aliquot of the completed PCR mix.
3. Add 2 µl of ExoProStar 1-Step to the reaction mix.
4. Incubate at 37°C for 15 min.
5. Incubate at 80°C for 15 min to inactivate the enzymes.

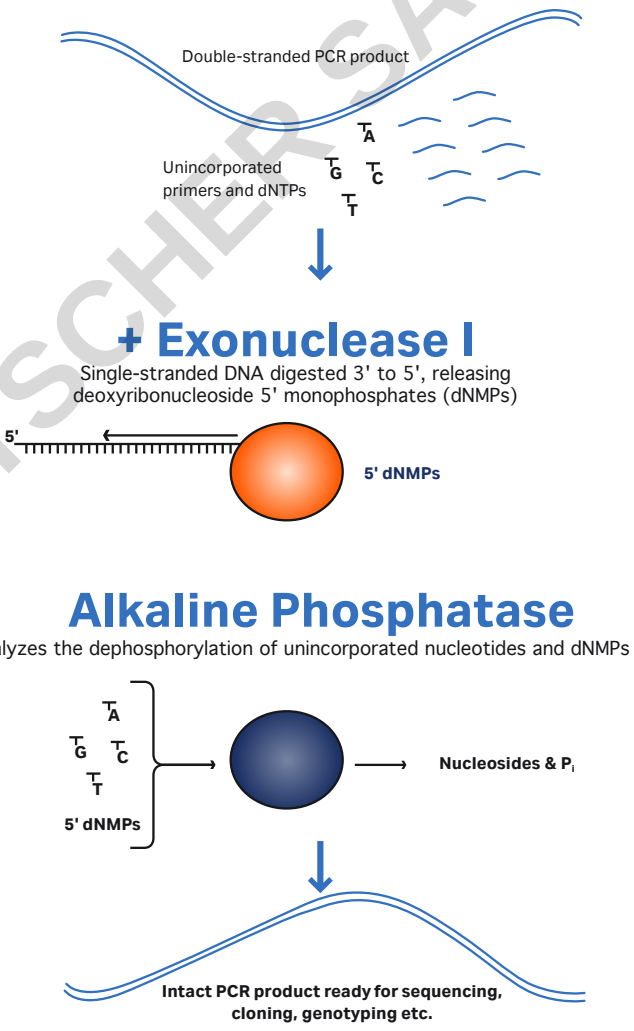


Fig 1. Schematic representation of the PCR cleanup process using ExoProStar 1-Step.

The PCR product is now ready for use in downstream reactions and processes. If a larger volume of PCR product is required, simply increase the volume of ExoProStar 1-Step added in proportion with the volume of PCR product.

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 1-Step was more efficient in digesting primers than the traditional USB® ExoSAP-IT® product when used under the manufacturer's standard operating protocol.

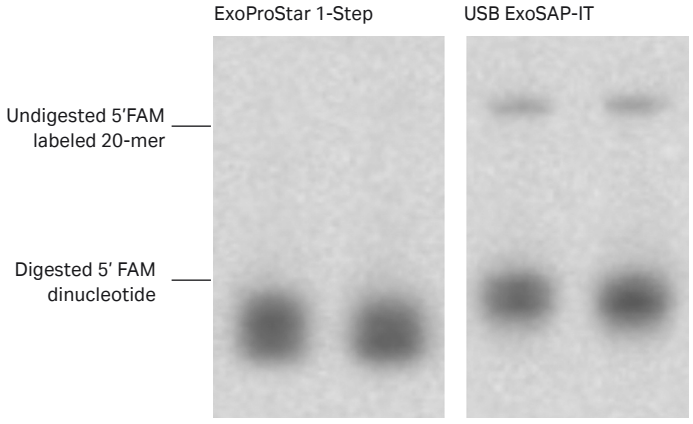


Fig 2*. Electrophoretic analysis of the digestion of a 5'FAM labeled 20mer primer. Reactions were conducted according to manufacturer's instructions for ExoProStar 1-Step and USB ExoSAP-IT respectively, using 10 pmol of primer per reaction. No detectable primer remained in the samples digested using ExoProStar 1-Step 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.

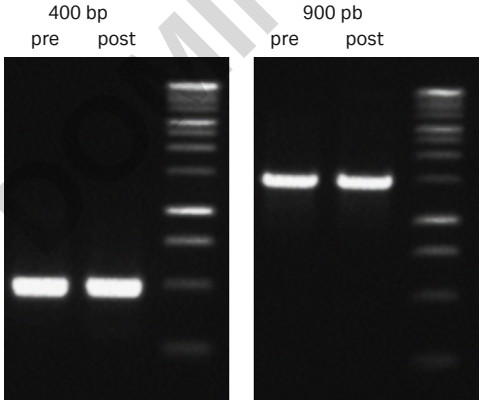


Fig 3. Agarose gel electrophoresis of different size PCR products pre- and post-digestion with ExoProStar 1-Step. Samples were digested for 15 min at 37° followed by denaturation of the ExoProStar 1-Step enzymes at 80°C for 15 min as per the recommended operating protocol. No loss of PCR product was 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 1-Step, Phred20 quality scores were routinely achieved at read lengths >800 bp, equivalent to or better than other approaches to sample preparation.

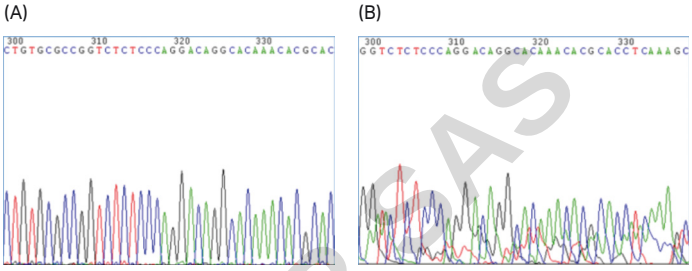


Fig 4. The importance of sample clean up before DNA sequencing is illustrated in the comparison between panel A, showing PCR sequence quality following treatment with ExoProStar 1-Step and panel B showing sequence quality without this treatment. Read length, base calling and sequence quality are significantly improved by the use of ExoProStar 1-Step.

Heat inactivation of ExoProStar 1-Step enzymes

Downstream operations can be adversely affected by the presence of active Exonuclease I or Alkaline Phosphatase in the PCR product following digestion. It is therefore essential that these enzymes 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.

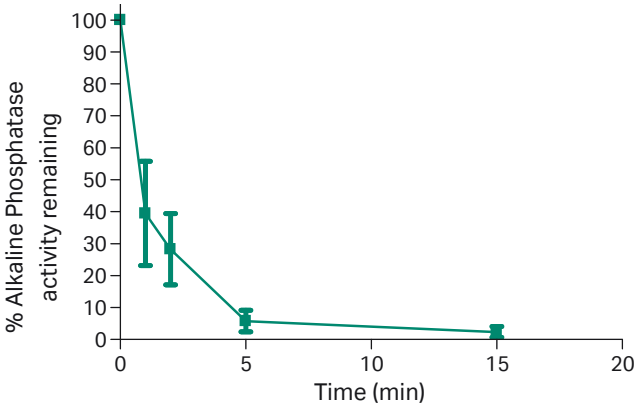


Fig 5. Temperature denaturation profile of Amersham Alkaline Phosphatase at 75°C showing rapid and complete denaturation within 15 min. The ExoProStar protocol recommends denaturation of the enzyme components at 80°C, providing even greater confidence in the inactivation of both enzymes prior to further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

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Kit components and storage

The ExoProStar 1-Step kit contains one tube of ready mixed Exonuclease I and Alkaline Phosphatase enzymes. The kit is supplied on dry ice and should be stored at -20°C. The product can be sub-aliquoted if required for storage convenience and should be maintained on ice during reaction set up.

Ordering information

ExoProStar 1-Step Enzymatic PCR and Sequence Reaction Cleanup Kit

Pack size	Code number
20 reactions	US77701
100 reactions	US77702
500 reactions	US77705
2000 reactions	US77720
5000 reactions	US77750

Related products

Amplification

Product	Quantity	Code number
dNTP set (100 mM each A,C,G,T)	4 × 100 µmol	28-4065-52
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 RTG™ (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

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

DNA cleanup

GFX™ PCR DNA and Gel 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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