GE Healthcare
Life Sciences

illustra™ ExoProStar™

illustra ExoProStar is optimized to purify PCR and sequencing set up reactions quickly, efficiently and reliably.

illustra ExoProStar contains illustra 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 GE Healthcare bio-Sciences Corp.

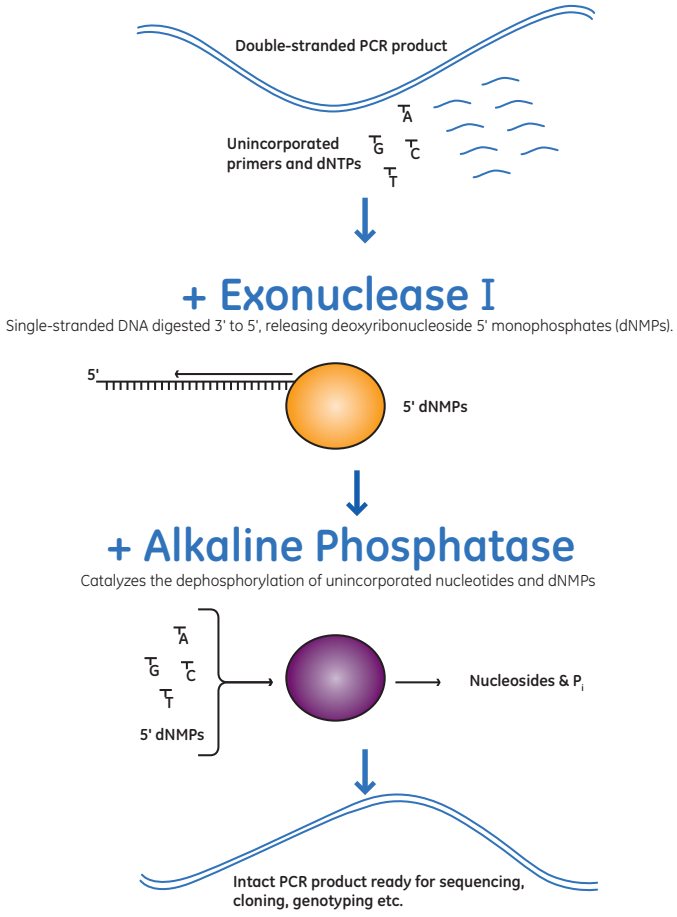

Fig 1. Schematic representation of the PCR cleanup process using illustra ExoProStar.

Optimized for efficient primer digestion

The new illustra 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, illustra ExoProStar was more efficient in digesting primers than the traditional USB® ExoSAP-IT® product when used under the manufacturer's standard operating protocol.

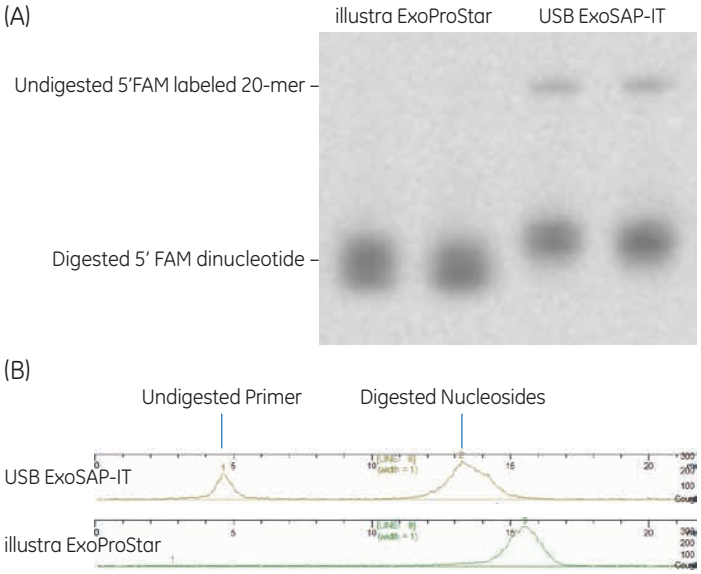

Fig 2. Panel A, Electrophoretic analysis of the digestion of a 5'FAM labeled 20mer primer. Reactions were conducted according to manufacturer's instructions for illustra ExoProStar and USB ExoSAP-IT using 10 pmol of primer per reaction. Panel B, Quantitation of digestion products conducted using a Typhoon™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

* Data presented in Fig. 2 was obtained by scientists at GE Healthcare, 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.

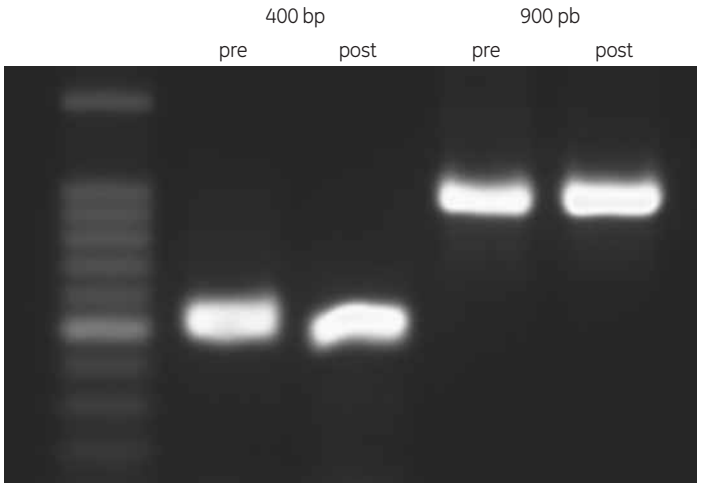

Fig 3. Agarose gel electrophoresis of different size PCR products pre- and post-digestion with illustra ExoProStar. Samples were digested for 15 min at 37° followed by denaturation of the illustra ExoProStar enzymes at 80°C for 15 min as per the recommended kit protocol. No loss of PCR product was detected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

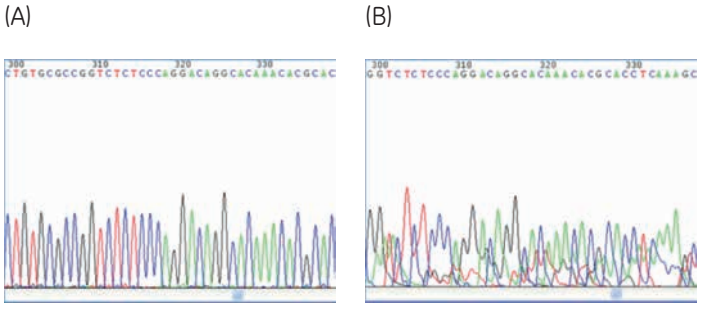

Fig 4. The importance of sample clean up before DNA sequencing is illustrated in the comparison between panel A, showing PCR sequence quality following treatment with illustra ExoProStar and panel B showing sequence quality without this treatment. Read length, base calling and sequence quality are significantly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

Heat inactivation of illustra 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 illustra Alkaline Phosphatase has been optimized to be quickly denatured, reducing the risk of downstream interference.

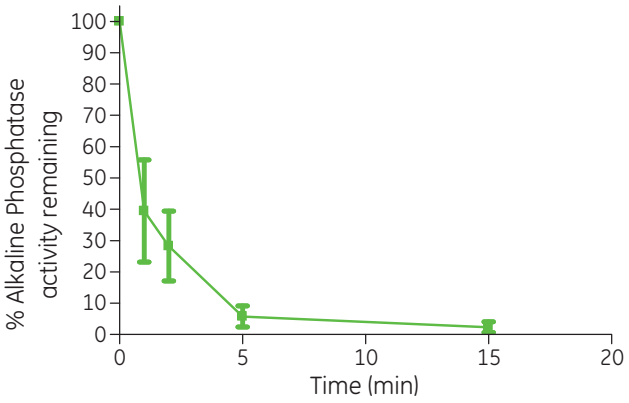

Fig 5. Temperature denaturation profile of illustra Alkaline Phosphatase at 75°C showing rapid and complete denaturation within 15 min. The illustra ExoProStar protocol recommends denaturation of the enzyme components at 80°C, providing greater confidence in the inactivation of both enzymes prior to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

Kit components and storage

The illustra ExoProStar kit contains one tube of illustra Exonuclease I and one tube of illustra 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

illustra 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
Illustra Ready-To-Go™ RT-PCR Beads (0.2 ml hinged tube with cap)	96 reactions	27-9259-01
Illustra PuReTaq™ Ready-To-Go PCR Beads (0.2 ml hinged tube with cap)	96 reactions	27-9559-01
Illustra 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

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

DNA cleanup

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

Enzymes

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

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