# Data file 29-0107-24 AB

# illustra™ ExoProStar™ 1-Step

illustra ExProStar 1-Step is optimized to purify PCR and sequencing set up reactions quickly, efficiently and relicibly, illustra ExpProStar 1-Step contains a mix of illustra Alkaline Phosphatase and Exonuclease I, formulated to work together to remove unincorporated primers and nucleatides from amplification reactions in preparation for sequencing, cloning, centrylar por Intel® 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

   East 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

illustra ExoProStar 1-Step builds on our long history and expertise in providing DNA cleanup products and expands on our ariginal patents for enzymatic sample cleanup using Exonuclease 1 and Alkaline Phosphatase. With illustra 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 illustra ExoProStar 1-Step requires only a single pipetting step, thanks to the premixed enzyme formulation.

#### Recommended protocol

- Remove the illustra ExoProStar 1-Step from the freezer and keep on ice while preparing the reaction.
   Take a 5 ul gliquot of the completed PCR mix.
- 3. Add 2 µl of illustra 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.





#### + Exonuclease I



### Alkaline Phosphatase



Fig 1. Schematic representation of the PCR cleanup process using illustra 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 illustra ExoProStar 1-Step added in proportion with the volume of PCR product.

# Optimized for efficient primer digestion The new illustra Alkaline Phosphatase and Examuelense I

enzymes have been optimized for highly efficient primer digestion, helping to improve the quality of downstream analysis. In analysis of primer digestion, flustra ExoProStar 1-Step was more efficient in digesting primers than the traditional USS® ExoSAP-IT® product when used under the manufacturer's standard operations protocol.

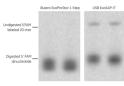


Fig 2: Beckrephoretic analysis of the digestion of a STAM labeled 20me primer Reactions were conducted according to manufacturer's instructions for illustra bis-Profiter 1-drep and USB BooGAPAT respectively, using 10 proof of primer per reaction. No detectable primer remained in the samples digested using illustra 6xxProfiter 1-drep by the dispersion of the primer remained in samples treated with USB 0xxSPAT.

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 ExcSAP-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 felt intact by the Exoruclease End Alkaline Phosphatase enzymes. The size of the PCR fragment does not offect the cleanue efficiency of the reaction.



Fig 3. Agarone gel electrophoresis of different size PCR products pre- and post-digestion with illustra ExaPoStar 1-Step. Samples were digested for 15 min at 37 followed by denaturation of the illustra ExaPoStar 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 illustra ExoProStar 1–Step, Phred20 quality scores were routinely achieved at read lengths >800 bp, equivalent to or better than other approaches to somple reprogration.

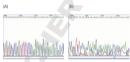


Fig 4. The importance of sample clean up before DNA sequencing is illustrated in the comparison between panel A, showing PCR sequence quality following treatment with illustra ExPrOStar 1-Step and panel B showing sequence quality without this treatment. Read length, base calling and sequence quality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

#### Heat inactivation of illustra ExoProStar 1-Step enzymes

Downstream operations can be adversely affected by the presence of active Exonuclease I or Alkaline Phasphatose in the PCR product following digestion. It is therefore essential that these enzymes are effectively denatured during the postdioestion heating step.

Same 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 aptimized to be quickly denatured, reducing the risk of downstream interference.

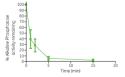


Fig 5. Temperature denaturation profile of illustra Akaline Phosphatase at 75°C showing rapid and complete denaturation within 15 min. The illustra ExploreStar protocol recommends denaturation of the enzyme components at 80°C, providing even greater confidence in the inactivation of both enzymes received for their denaturations.

### Kit components and storage

The illustra ExpProStar 1-Step kit contains one tube of ready mixed illustra 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

#### illustra ExoProStar 1-Step Enzymatic PCR and Sequence Reaction Cleanup Kit

Pack size	Code num	be
20 reactions 100 reactions 500 reactions 2000 reactions 5000 reactions	US7770 US7770 US7770 US7772 US7773	12

## Related products

#### Amplification

Product	Quantity	Code number
dNTP set (100 mM each A.C.G.T)	$4 \times 100  \mu mol$	28-4065-52
Illustra Ready-To-Go <sup>TM</sup> 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		

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

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 Soin 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	$10 \times 96$ 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
Wanted Control of Control	25.00 14-	F700777

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www.gelifesciences.com/illustraExoProStar

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First published Nov. 2011.

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29-0107-24 AB 01/2013