Single-Stranded DNA Removal Kit

Introduction

BcMag™ Single-Stranded DNA Removal Kit uses magnetic microspheres covalently conjugated with a high purity of Exonuclease I (ExoI) to remove single stranded DNA from the solution. ExoI is derived originally from E. coli. This enzyme is a 55 kDa exonuclease that hydrolyzes single-stranded DNA (ssDNA) stepwise in a 3'→5' direction. This Exonuclease is highly specific for single-stranded DNA and does not react with double-stranded DNA or RNA and DNA strands with terminal 3'-OH groups blocked by phosphoryl or acetyl groups. Exonuclease I is tolerant of a wide range of buffer conditions and can typically be added to PCR reactions**.** The Exonuclease I can be inactivated by heat treatment at 80°C for 15 minutes. The magnetic bead immobilized with ExoI can efficiently remove single-stranded DNA such as PCR primer from reactions with no nucleases remaining in the solution due to the nuclease stably and covalently immobilized with the magnetic beads.

Applications

- Presequencing clean-up for PCR products
- Megaprimer PCR for site-directed mutagenesis
- Removal of ssDNA containing a 3'-hydroxyl terminus from nucleic acid mixtures
- Assay for the presence of single-stranded DNA with a 3'-hydroxyl terminus

Features and Advantages

- Efficient one-tube and extraction-free protocol (Fig.1).
- Ultrafast: Process 96 samples in less than 30 minutes with <10-second Hands-on Time
- Nuclease recovered at the end of the reaction thereby can be reused.
- Easy separation of the nuclease from the reaction.
- Stability of the immobilized nuclease increases.
- Cost-effective: Eliminates columns, filters, laborious, organic reagents, and minimal plasticware required.
- High throughput: Compatible with many different automated liquid handling systems.

Workflow

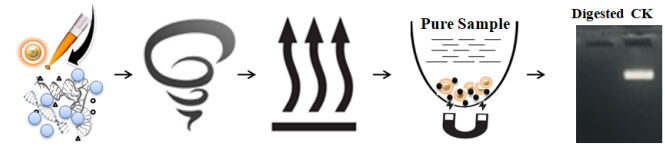

Fig.1

Formulation: Liquid (Supplied in 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50 % (v/v) glycerol)

Activity: 1 µl Magnetic Beads will digest 0.5µg of oligonucleotides in 67 mM glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 1 mM DTT in 30 minutes at 37 °C.

Reaction buffer: 10X Reaction Buffer 670 mM glycine-KOH (pH 9.5 at 25 °C), 67 mM $MgCl_2$,10 mM DTT.

Shipping: Shipped at ambient temperature. Upon receipt, store magnetic nuclease beads at -20°C. Aliquot to avoid repeated freezing and thawing.

PROTOCOL

A. Accessory equipment

Magnetic Rack

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02)

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	• BcMag Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag 96-well Plate Magnetic Rack.	• BcMag 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-05)

B. Procedure

- Do not use buffers containing organic solvents.
- Typically, the bead is added directly into any standard buffer at the desired amount of the beads based on the concentration of the ssDNA (1 µl Magnetic Beads will digest 0.5µg of oligonucleotides)

1. Shake the bottle to resuspend the Magnetic beads until it is homogeneous entirely.

   IMPORTANT! It is essential to mix the beads before dispensing. Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 2 minutes.
2. Add an appropriate amount of the magnetic beads to a reaction.
3. Mix the sample with beads for 1-2 minutes by slowly pipetting up and down 20-25 times or Vortex the sample for 2 minutes at 2000 rpm.
4. Incubate at 37°C with continuous rotation for 30 minutes.
5. Place the sample plate or tube on the magnetic Rack for 30 seconds or until the solution is clear.
   (Option: centrifuge at 3500 rpm for 45 seconds)
6. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications.

Related Products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

Phone: +1-858-909-0079 **Bioclone Inc**. Fax: +1-858-909-0057