One-Step PCR Inhibitor Removal Kit

PCR technology has become one of the most potent molecular biological tools in the last few decades. However, biological samples collected from different materials often contain various PCR inhibitors that can interfere with PCR amplification. PCR inhibitors are a diverse group of compounds that inhibit the amplification of DNA through the polymerase chain reaction (PCR). PCR inhibitors generally exert their effects by directly binding the active site of a DNA polymerase to cause decreased sensitivity or complete failures of the DNA amplification. Therefore, removing PCR inhibitors from the DNA extracts before the PCR amplification is vital for all downstream applications.

Where Do the PCR inhibitors originate?

PCR inhibitors exist in a variety of biological materials (organs, blood, body fluids, etc.), environmental samples (water, soil, air, etc.), and food (meat, milk, fruits, vegetables, seafood, etc.). In addition, inhibitory substances may be accidentally added during transport, sample processing (e.g., pre-concentration procedures), or nucleic acid extraction. PCR inhibitors can come from the original sample themself, sample preparation, DNA purification process, or dirty plasticware. Table1 shows some examples of sources and their specific inhibitors. Table 1 lists the common PCR inhibitors.

Sources	Inhibitor
Blood, Muscle tissues	Heparin, Hemoglobin, Immunoglobulins, Proteases, Nucleases, Acyclovir, Hormones, IgG, Lactoferrin, Myoglobin, Collagen
Milk	Proteases, Calcium ions
Stool	Bile salts, Complex polysaccharides, Lipids, Urate
Plants	Pectin, Polyphenols, Polysaccharides, Xylan, Chlorophyll
Soil	Fulmic acids, Humic acids, Humic material, Metal ions, Polyphenol
Sample preparation	KCl, SDS, Xylene
DNA purification	Chaotropic salts, Ethanol, Isopropanol, Phenol, Sodium acetate
Dirty plasticware	Protease, Nucleases

Table1. PCR inhibitor

How to remove the PCR Inhibitors?

Several methods have been used for the removal of inhibitors or the reduction of their effects.

1. Dilute the sample extracts containing PCR inhibitors. It is simple, but the diluted DNA may not be enough for successful DNA amplification, and a decrease in sensitivity accompanies the dilution.
2. Chelex such as Chelex1-100 and Phenol–Chloroform-based protocols. Both methods are inefficient for removing most of the PCR inhibitors since Chelex can only remove some divalent ions. At the same time, the Phenol–Chloroform method can only deplete lipids and proteins.
3. Column chromatography such as Sephacryl S-400, Sephadex G-200, and silica-based spin-column or magnetic beads effectively remove some of the inhibitors, but the process is labor-intensive and time-consuming.

In summary, although these methods can remove some PCR inhibitors, however, practically they have the following limitations such as:

1. Less efficient, time-consuming, or labor-intensive.
2. Potential loss of DNA sample during processing.
3. Chaotropic salt and ethanol are potentially carried over into the eluted DNA.
4. They are not suitable for high-throughput processing and automation.

For this reason, Bioclone developed a novel, highly efficient PCR inhibitor removal system based on magnetic beads.

BcMag™ One-Step PCR Inhibitor Removal Kit provides one-step removal of PCR inhibitor from impure DNA samples before PCR, RT, and other downstream applications based on negative chromatography. The magnetic beads are superparamagnetic and modified with our proprietary chemistry. When mixed with inhibitor-containing samples, the beads instantly capture and remove the PCR inhibitors. At the same time, only the pure DNA remains in the solution and is ready for all downstream applications (Fig.1). The beads can

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effectively remove many common inhibitors such as polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, heparin, detergents, and denim dyes, and divalent cations such as Ca^{2+}, Mg^{2+}, etc.

Workflow

The protocol is straightforward and fast: one tube, one step, and one minute (Fig.1). Add the magnetic beads directly to the pre-purified DNA samples and vortex or pipette to capture and remove the impurities. After vortexing/pipetting, the beads are magnetically removed, while the supernatant contains the purified and ready-to-run products. Unlike standard bind-wash-elute protocol, this convenient procedure does not contain traces of organic solvents, chaotropic salts, or EDTA and is almost 100% DNA recovery. The beads enable 96 samples to be processed simultaneously in less than 10 minutes with cost-effective lab vortex mixers.

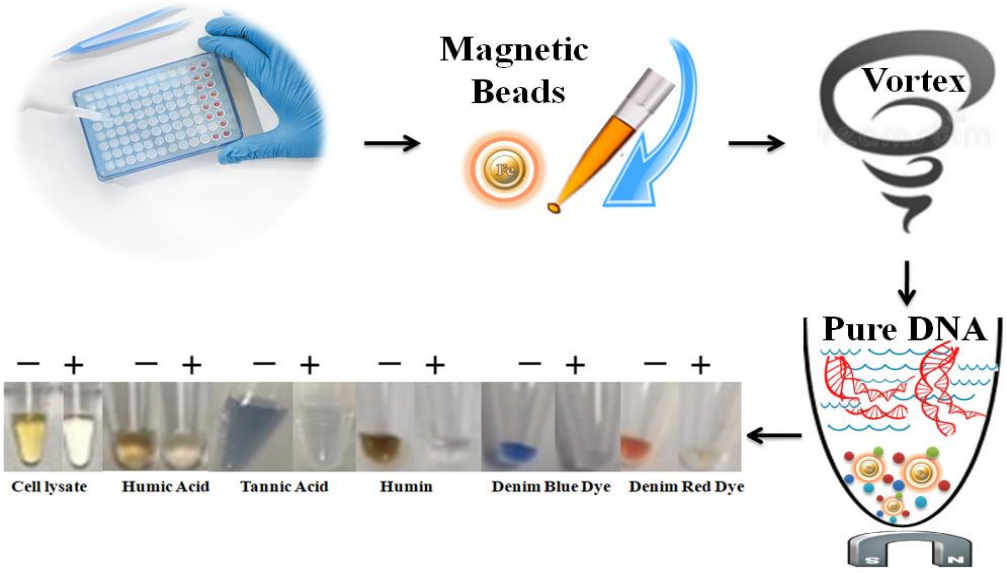

Fig.1 Workflow for PCR inhibitor removal

Features and Advantages:

- Simple protocol: No liquid transfer, One-tube, One-step
- Ultrafast: One-minute manual protocol or less than 10-minutes vortex (96 samples)
- Higher purity and recovery > 90% DNA (> 50bp).
- Effective Cleanup: polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, detergents, divalent cations such as Ca^{2+}, Mg^{2+}, etc.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and ethanol
- High throughput: Compatible with many different automated liquid handling systems

Handling and Storage:

- Store at 4°C upon arrival for up to 12 months.
- DO NOT FREEZE

Products

Products	Catalog # AX-101	Catalog # AX-102
BcMag™ One-Step PCR inhibitor removal Kit	1 ml	2.5 ml

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Protocol

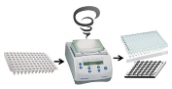
The following protocol is an example. The beads and sample volume can be rational Scale-up *(*or down) as needed. Do not use buffers containing organic solvents.

Equipment

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) • 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-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ****** *IMPORTANT!* If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	
Addition items are required if using 96-well microplates	
Fisher Scientific™ Microplate Advanced Vortex Mixers	Fisher, Cat#:02-216-101
OHAUS Microplate Vortex Mixers	OHAUS, Cat#:30392160
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed≥ 800 rpm	
Clear Flat-bottom Non-Binding Assay Microplates	

Procedure

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 10µl magnetic beads to a 100 µl pre-purified DNA sample.
   IMPORTANT! Users need to optimize the ratio of beads, and the concentration of the PCR inhibitors since the concentration of the inhibitors varies from sample to sample.
3. Mix the sample with beads for 1 minute by slowly pipetting up and down 20-25 times or by vortex mixer at 2000 rpm for 5 minutes.



4. Place the sample plate or tube on the magnetic separation plate for 30 seconds or until the solution is clear.
5. 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.

General references

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1. Poddar SK, Sawyer MH, Connor JD. Effect of inhibitors in clinical specimens on Taq and Tth DNA polymerase-based PCR amplification of influenza A virus. J Med Microbiol. 1998 Dec;47(12):1131-5.
2. Kaltenboeck B, Wang C. Advances in real-time PCR: application to clinical laboratory diagnostics. Adv Clin Chem. 2005;40:219-59.
3. Alaeddini R. Forensic implications of PCR inhibition--A review. Forensic Sci Int Genet. 2012 May;6(3):297-305.
4. Al-Soud WA, Rådström P. Purification and characterization of PCR-inhibitory components in blood cells. J Clin Microbiol. 2001 Feb;39(2):485-93.

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

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