One-Step PCR Cleanup Kit

PCR purification is a method for removing DNA or RNA that has undergone a polymerase chain reaction (PCR) amplification (PCR). Contaminants from the PCR process, including salts, enzymes, and primer dimers, must be removed. Following that, additional uses, such cloning or sequencing, can be made of the purified DNA or RNA.

BcMag™ One-Step PCR Cleanup Kit is a specially designed kit for ultrafast and efficient purification of post PCR or other DNA reactions. The protocol is not only straightforward (one tube and one step, as shown in Fig.1) but also very flexible in removing different size DNA fragments by adjusting processing time, buffer's pH, and detergent concentration (table1). The magnetic Beads are added directly to the finished PCR reactions or other DNA reactions and mixed by a vortex mixer or pipetting to capture and remove the impurities (e.g., excess primer, dimer, adapter, salt, detergent, dNTPs, and enzyme). After mixing, the beads are magnetically removed, while the supernatant contains the purified and ready-to-run products. In just 1 minute, the purified DNA is ready for downstream applications, such as Sanger Sequencing, Restriction Digestion, Cloning, SNP Detection, or Library Preparation for NGS. The beads enable 96 samples to be processed simultaneously in less than 10 minutes.

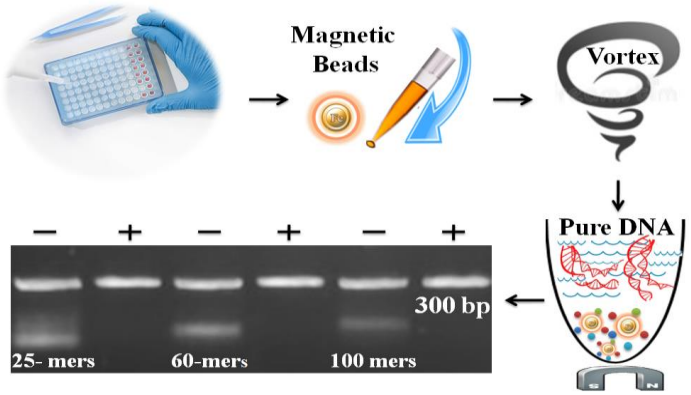

Fig.1 One-Step PCR Cleanup Kit

Features and Advantages:

- Simple protocol: No liquid transfer, One-tube, One-step
- Ultrafast: One-minute protocol
- Higher purity and recovery > 90% DNA.
- Effective Cleanup: Removes excess primer (<100- mer ssDNA), dimer, adapter, a salt such as Mg^{2+}, detergent, dNTPs, enzymes, and dye.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and ethanol
- High throughput: Compatible with many different automated liquid handling systems

Products

Components	Storage	100 preps, Cat #: AP-101	250 preps, Cat #: AP-102
BcMag™ One-step PCR Cleanup Kit	4°C	500 µL	1.25 mL

PROTOCOL

A. Materials Required by the User

- 18.2 MΩ.cm, DNase/RNase-Free Ultrapure Water
- Triton™ X-100, Sigma, Catalog # T8787
- Others

Item	Source

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

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!*** Using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates must be ≥2.5mm.	

B. Procedure

Important!

1. The following protocol is optimized for the efficient cleanup of 10µl DNA sample. The procedure may need to be optimized if an alternative reaction scale is used.
2. Shake or vortex the bottle to completely resuspend the magnetic beads before using.
3. Do not allow the magnetic beads to sit for more than two minutes before dispensing.
4. Based on applications, the user should choose buffer conditions based on table 1. For example, if the sample does not contain detergent, add 1 µL of 1% Triton™ X-100 solution to a 10 µL sample (final concentration is 0.1%).
5. Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green. OD260 methods such Nanodrop and UV-spectrophotometry are not-suitable.

Table 1

DNA Fragment Removal						
Buffer / DNA	+ 0.1% Triton x-100, pH7.5	- 0.1% Triton x-100 pH7.5	+ 0.1% Triton x-100 pH 8.0	- 0.1% Triton x-100 pH 8.0	+ 0.1% Triton x-100 pH 8.8	- 0.1% Triton x-100 pH 8.8
dsDNA (100 bp)	No removal	removal	removal	removal	No removal	removal
dsDNA (150 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (200 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (300 bp)	No removal	No removal	No removal	No removal	No removal	No removal
ssDNA 100 mer	removal	removal	removal	removal	removal	removal
dsDNA- Double-Stranded DNA; ssDNA- Single-stranded DNA The assay was done by using the following conditions: 1. 10 mM Tris-HCl with or without 0.1% triton (final concentration) and three different: pH 7.5, pH 8.0 and pH 8.8						

1. Add 5 µL magnetic beads to the 10 µL DNA sample.

2. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.

3. Mix thoroughly for 1 minute by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2500 rpm.

4. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.

5. Place the sample plate on the magnetic separation plate for 30 seconds or until the solution is clear to separate beads from the solution.

6. Transfer the supernatant to a clean plate while the sample plate remains on the magnetic separation plate for downstream applications.

C. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA Recovery	Vertexing speed is too fast. Vertexing time is too long.	• Reducing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, has to be optimized.
	Using too many magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.
Failure to remove impurities.	Used inappropriate PCR tubes or PCR plates	Make sure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates is ≥2.5mm.
	Vortex speed is too slow, or vortex time is too short.	• Increasing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, has to be optimized.
	Using fewer magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.
	Strong secondary structure of DNA fragments (<50bp dsDNA or < 100 mer ssDNA)	Denature the sample by heating it at 95°C for 2 min.
	Too much primer, dimer, adaptor, free dye, and detergent	• Use more magnetic beads. • Perform the second round of purification by following the same protocol.

Related Product

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