AFTSpin Universal DNA Purification Kit

Cat. No. : RK30100

Product components

Components	Component number	Size 50 RXN	Size 200 RXN
Buffer DB	RM30102	38 mL	2 × 75 mL
Buffer BL	RM30101	5 mL	20 mL
Spin Column 1	RM30180	50 pk	200 pk
Collection Tube 2 mL	RM30190	50 pk	200 pk
Buffer WB*	RM30110	12.5 mL	2 × 25 mL
Buffer EB	RM30111	5 mL	20 mL

*Note: Add 50 mL of absolute ethanol (for size 50 RXN) to Buffer WB. Add 100 mL of absolute ethanol (for size 200 RXN) to each Buffer WB bottle prior to initial use (Final volume of each Buffer WB bottle is 125 mL).

Product Description

This kit uses a DNA purification column to recover DNA fragments from agarose gel or to directly purify PCR products. Under specific conditions, the DNA fragments selectively bind to the silicon matrix membrane in the spin column, allowing for impurities such as primers, nucleotides, proteins, and enzymes to be removed by Wash Buffer through a series of rapid washing and centrifugation steps. The purified DNA is eluted from the silicon matrix membrane with low salt and high pH Elution Buffer. Up to 15 μg of DNA fragments, 100 bp-8 kb in length, can be recovered using this kit, with the recovery rate up to 80%.

Storage

This kit is stable for 12 months when stored at room temperature. Minor precipitation of reagents does not affect experimental results. If necessary, reagents can be placed in a water bath at 37℃ for 10 minutes to dissolve precipitate.

Highlights

1. This kit uses a high-quality gel solubilization buffer which, unlike traditional gel solubilization solution, does not contain sodium iodide and perchlorate, and as such does not inhibit downstream reactions such as digestion and ligation-dependent cloning after recovery.
2. The unique gel solubilization/binding buffer formula unifies both gel solubilization and binding steps, allowing the kit to be used in many situations such as agarose DNA recovery, PCR product purification, and digestion product purification and recovery. The gel solubilization/binding buffer is a distinct yellow color, which allows for visual detection of gel solubilization and monitoring of pH value shift during sample handling, giving users the ability to optimize best binding and greatly improve recovery efficiency.
3. The kit is fast and convenient, without requiring the use of toxic reagents such as phenol and chloroform or ethanol precipitation.
4. The recovered and purified DNA fragments range between 100 bp and 40 kb in length, and recovery of excessively long or short fragments is diminished.

Precautions

1. All solutions should be clear. While minor precipitation will not affect the efficiency of the kit, if significant precipitation is visible, the solution can be heated to 37℃ in a water bath for a few minutes to dissolve precipitate. All reagents should be brought to room temperature before use.
2. Avoid volatilization, oxidation and pH changes caused by extended exposure of reagents to air. Tightly close all reagent bottles immediately after use.

3. Buffer BL is a strong alkaline solution; wear proper PPE and avoid direct contact with skin. Buffer DB contains irritating compounds. Wear latex gloves when operating to avoid contamination with skin, eyes, and clothes. Should contact with skin or eyes occur, to immediately flush with copious amounts of water or physiological saline rinse and seek timely medical treatment.
4. All centrifugation steps should be completed at room temperature using a benchtop centrifuge at rotation speed of 12,000 rpm (~13,400 x g).
5. The amount and final concentration of DNA recovered depends on the amount of starting DNA, the size of DNA fragments, and upon on the volume of Elution Buffer used and the time Elution Buffer incubates on the column.
6. During gel extraction, exposure to ultraviolet light should be limited as much as possible to prevent unwanted degradation of DNA, especially larger fragments.
7. Water can be used to elute samples instead of Elution Buffer as long as the pH of the water used is greater than pH7.5 as low pH will affect elution efficiency. DNA fragments eluted with water should be stored at -20°C. DNA fragments may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) if long-term storage is required, but EDTA may affect downstream digestion reactions and should be diluted appropriately when used.
8. Prior to the initial use, add the specified amounts of absolute ethanol to the Buffer WB and mix thoroughly. After adding, mark the reagent bottle to indicate that ethanol has been added, to avoid multiple additions!

Operational Instructions

Agarose gel recovery

1. To equilibrate Spin Column 1, insert a new Spin Column 1 into a Collection Tube and pipette 100 μL of Buffer BL onto the column membrane. Centrifuge at 12,000 rpm (~13,400 x g) for 1 minute, discard the waste from the collection tube and return the spin column to the collection tube.
2. Under a long-wave length UV lamp, slice the DNA band to be recovered from the gel with a clean blade, and remove excess gel as much as possible.
3. Weigh the gel, add Buffer DB at 3 times the volume of the gel sample. (i.e. For a gel sample weighing 100 mg, its volume can be considered 100 μL and thus 300 μL of Buffer DB should be added.)

   Note: If the agarose gel concentration is greater than 2%, increase Buffer DB to 6 times the volume of the sample.
4. Incubate the sample at 56°C for 10 minutes until the gel completely dissolves and the color becomes uniform. Vortex every 2-3 minutes to help accelerate dissolution.
5. Optional step: Add 150 μL of isopropanol per 100 mg of initial gel weight and shake to mix well. Sometimes the addition of isopropanol can improve the recovery rate for DNA fragments smaller than 4 kB. Do not centrifuge after addition. When fragments larger than 4 kb are to be recovered, isopropanol should not be added.
6. Add the melted gel solution to Spin Column 1, placed in a collection tube, and allow to stand at room temperature for 1 minute. Centrifuge at 12,000 rpm (~13,400 x g) for 30-60 seconds, then discard the waste in the collection tube.

   Note: If the total sample volume exceeds 750 μL, the solution may be divided and added to the same spin column in 2 subsequent centrifugation steps. After mixing the filtered Buffer DB with residual Buffer BL in the collection tube, the solution may change from yellow to orange or even purple, which is the normal color change of the phenol red PH indicator under alkaline conditions.
7. Add 600 μL of Buffer WB (**check that absolute ethanol has been added**), centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds and discard the waste.
8. Add 600 μL of Buffer WB, centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds and discard the waste.
9. Place the Spin Column 1 into an empty collection tube and centrifuge at 12,000 rpm (~13,400 x g) for 2 minutes to remove Buffer WB as much as possible, so as to avoid inhibiting the downstream reaction by residual ethanol in Buffer WB.
10. Remove the Spin Column 1 into a clean centrifuge tube, add 50 μL of Buffer EB (preferably heated in a 65-70°C water bath in

advance) to the center of the spin column membrane, and allow to stand at room temperature for 2 minutes. Centrifuge at 12,000 rpm (~13,400 x g) for 1 minute. To increase final sample concentration, the resulting solution can be re-added to the same Spin Column1 and centrifuged again for 1 minute. The resulting liquid contains highly purified DNA.

Note: The larger the elution volume, the higher the elution efficiency. If a higher concentration of DNA is required, the elution volume can be appropriately reduced to a minimum volume of not less than 25 μL. Smaller elution volumes can reduce elution efficiency and DNA yield.

DNA purification such as PCR products or enzyme fragments

1. To equilibrate Spin Column 1, insert a new Spin Column 1 into a Collection Tube and pipette 100 μL of Buffer BL onto the column membrane. Centrifuge at 12,000 rpm (~13,400 x g) for 1 minute; discard the waste from the collection tube and return the spin column to the collection tube.
2. Add 500 μL of Buffer DB per 100 μL of PCR amplification products or digestion products and mix thoroughly. (If the initial system is less than 100 μL, adjust to 100 μL with double-distilled water in advance; if the volume of PCR products or digestion products is greater than 100 μL, the amount of Buffer DB used can be increased in equal proportion).
3. Place the pretreated Spin Column 1 placed in a collection tube. Add the solution obtained in the step 2 to the membrane and allow to stand at room temperature for 1 minute. Centrifuge at 12,000 rpm (~13,400 x g) for 30-60 seconds, then discard the waste in the collection tube.

   Note: If the total volume exceeds 750 μL, the solution can be added to the same Spin Column 1 in separate steps. When the filtered Buffer DB is mixed with the remaining Buffer BL in the collection tube, the solution may change from yellow to orange-red or even purple, which is the normal color change under alkaline conditions of the phenol red PH indicator.
4. Add 600 μL of Buffer WB (**check that absolute ethanol has been added**), centrifuge at 12,000 rpm (~13,400 x g) for 30 seconds, then discard the waste.
5. Add 600 μL of Buffer WB, centrifuge at 12,000 rpm for 30 seconds, then discard the waste.
6. Place the Spin Column 1 into an empty collection tube and centrifuge at 12,000 rpm (~13,400 x g) for 2 minutes to remove Buffer WB as much as possible, so as to avoid inhibiting the downstream reaction by residual ethanol in Buffer WB.
7. Move the Spin Column 1 into a clean centrifuge tube and add 50 μL of Buffer EB to t**he center of the spin column membrane** (preferably heated in a 65-70°C water bath in advance). Allow to stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm (~13,400 x g) for 1 minute. To increase final sample concentration, the resulting solution can be re-added to the same Spin Column 1 and centrifuged again for 1 minute. The resulting liquid contains highly purified DNA.