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Product Information

GenElute™ PCR Clean-Up Kit

Catalog Number **NA1020**

TECHNICAL BULLETIN

Product Description

The GenElute PCR Clean-Up Kit is designed for rapid purification of single-stranded or double-stranded PCR amplification products (100 bp to 10 kb) from the other components in the reaction such as excess primers, nucleotides, DNA polymerase, oil, and salts. This kit combines the advantages of silica binding with a microspin format and eliminates the need for expensive resins or toxic organic compounds such as phenol and chloroform.

DNA purification is achieved in a few easy steps. First, DNA is bound on a silica membrane within the spin column. The bound DNA is washed and the clean, concentrated DNA is eluted in the buffer of choice. Each column can purify up to 100 µL or 10 µg of PCR amplified DNA and recover up to 95% of PCR products between 100 bp and 10 kb. More than 99% of the primers and most primer-dimers (<40 bp) are removed. Purified DNA can be used in enzymatic reactions, conventional or automated sequencing, cloning, and microarray analysis.

Components/Reagents

Reagents Provided (Sufficient for 70 purifications)	**Catalog Number**	**Quantity**
Column Preparation Solution	C2112	60 ml
Binding Solution	B7556	40 ml
Wash Solution Concentrate	W3637	12 ml
Elution Solution	E7777	8 ml
GenElute plasmid mini spin column	G6415	70 each
Collection Tubes, 2 ml	T5449 or T7813	2 x 70 each

Equipment and Reagents Required But Not Provided

- Ethanol (absolute), Catalog Numbers E7023 or 459836
- Microcentrifuge
- Microcentrifuge tubes
- Water, Molecular Biology Reagent, Catalog Number W 4502

Precautions and Disclaimer

The GenElute PCR Clean-Up Kit is for R&D use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Preparation Instructions

1. **Thoroughly mix reagents**. Examine the reagents for precipitation. If any reagent has formed a precipitate, warm at 55-65 °C until the precipitate dissolves and allow to cool to room temperature before use.

2. **Wash Solution:** Dilute the Wash Solution Concentrate with 48 ml of 100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of ethanol.

Storage/Stability

Store the kit at room temperature. If any kit reagent forms a precipitate upon storage, see Preparation Instructions.

Procedure

All centrifugations (spins) are at 12,000 – 16,000 x *g* (See Appendix I to convert *g*-force to RPM).

1. Insert a GenElute plasmid mini spin column (with a blue o-ring) into a provided collection tube, if not already assembled. Add 0.5 ml of the Column Preparation Solution to each mini spin column and centrifuge at 12,000 x *g* for 30 seconds to 1 minute. Discard the eluate.

Note: The Column Preparation Solution maximizes binding of the DNA to the membrane resulting in more consistent yields.

2. Add 5 volumes of Binding Solution to 1 volume of the PCR reaction and mix. For example, add 500 µL of Binding Solution to 100 µL of the PCR reaction. Transfer the solution into the binding column. Centrifuge the column at maximum speed (12,000-16,000 x*g*) for 1 minute. Discard the eluate, but retain the collection tube.

3. Replace the binding column into the collection tube. Apply 0.5 ml of diluted Wash Solution to the column and centrifuge at maximum speed for 1 minute. Discard the eluate, but retain the collection tube.

Note: Be sure to add ethanol to the Wash Solution Concentrate prior to first time use. See Preparation Instructions.

4. Replace the column into the collection tube. Centrifuge the column at maximum speed for 2 minutes, without any additional wash solution, to remove excess ethanol. Discard any residual eluate as well as the collection tube.

5. Transfer the column to a fresh 2 ml collection tube. Apply 50 µL of Elution Solution or water to the center of each column. Incubate at room temperature for 1 minute.

Note: When eluting with water, make sure that the pH of the water is between 5.5 and 8.5. Elution may also be performed using the Elution Solution diluted 10-fold with water.

6. To elute the DNA, centrifuge the column at maximum speed for 1 minute. The PCR amplification product is now present in the eluate and is ready for immediate use or storage at –20 °C.

Troubleshooting Guide

Problem	Cause	Solution
DNA recovery is low.	Wash solution was not diluted properly.	Make certain ethanol was added to the Wash Solution Concentrate and the cap is replaced after each use to prevent evaporation (see Preparation Instructions).
	DNA was not eluted properly.	DNA must be eluted with a low salt solution such as the Elution Solution or water.
		Add the Elution Solution onto the center of the filter.
		Allow the Elution Solution to incubate in the column for one minute.
Performance in downstream enzymatic applications is poor.	DNA eluate is contaminated with salt.	Do not allow the binding or wash flow-through liquid to come in contact with the bottom of the binding column following the spin steps.
	Eluate is contaminated with ethanol, which was not entirely removed before elution.	Be sure to centrifuge at maximum speed for 2 minutes in step 4 of the Procedure.

Related Products	Catalog Number	Related Products	Catalog Number
Extract-N-Amp™ Plant PCR Kits	XNAR XNAP2 XNAP2E XNAP2RE	JumpStart REDTaq® DNA Polymerase	D8187
		JumpStart REDTaq ReadyMix™ PCR Reaction Mix	P0982
Extract-N-Amp Blood PCR Kits	XNAB2 XNAB2R XNAB2E XNAB2RE	JumpStart REDAccuTaq® LA DNA Polymerase Mix	D1313
		REDAccuTaq LA DNA Polymerase	D4812
		Enhanced Avian HS RT-PCR Kit	HSRT100
AccuTaq™ LA DNA Polymerase	D8045		
Taq DNA Polymerase	D1806 D4545	Enhanced Avian First Strand Synthesis Kit	STR1
KlenTaq® LA DNA Polymerase Mix	D5062	Enhanced Avian Reverse Transcriptase	A4464
JumpStart™ Taq DNA Polymerase	D9307 D4184		

Appendix I

Note: All centrifugation speeds are given in units of g. Please refer to Table 1 for information on converting g-force to rpm. *If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time proportionally. Spin until all liquid passes through the column.*

Table 1. Conversion of Centrifugal Force (in units of *g*) to RPM for Common Rotors

Centrifuge	Rotor	Tubes (max)	Radius (cm)	RPM at 12,000 x *g*	RPM at 16,000 x *g*
Eppendorf					
5410		12	5.8	13,555	15,652
5415C	F45-18-11	18	7.3	12,124	14,000
5415D&R	F45-24-11	24	8.3	11,392	13,155
5417C,D,&R	F45-30-11	30	9.5	10,634	12,279

See table above for spin speeds in rpm for selected common centrifuges and rotors. The correct rpm for unlisted rotors can be calculated using the formula:

$$RPM = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*; *r* = radius of the rotor in cm; *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

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