GenElute™ PCR DNA Purification Kit

Product Code **GEN-PCR**
Technical Bulletin MB-595

PRODUCTINFORMATION

TECHNICAL BULLETIN

Product Description
The GenElute™ PCR DNA Purification Kit is designed for rapid purification of single-stranded or double-stranded PCR‡ amplification products (100 bp to 10 kb) from other components in the reaction such as excess primers, nucleotides, DNA polymerase, oil, and salts. This kit combines the advantages of a silica-based system 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 then the clean, concentrated DNA is eluted in the buffer of choice. Each column can purify up to 10 µg of PCR amplified DNA and recover up to 95% of fragments between 100 bp to 10 kb in size. More than 99% of the primers and most primer-dimers (<50 bp) are removed. Purified DNA can be used in enzymatic reactions, conventional or automated sequencing, blotting, and *in situ* hybridization.

Reagents Provided (Sufficient for 70 purifications)	**Product Code**	**Quantity**
Binding Solution	B 2911	45 ml
Wash Solution Concentrate	W 4127	15 ml
Binding Column	G 2416	70 each
Collection Tubes, 2 ml	T 7813	2 x 70 each

Equipment and Reagents Required But Not Provided
- Ethanol (95-100%), Product Code E 7148 or E 7023
- Microcentrifuge
- Microcentrifuge tubes
- TE (Tris-EDTA) buffer, prepared from 100X TE, Product Code T 9285 **OR**
- Molecular Biology Reagent Water, Product Code W 4502

Precautions and Disclaimer
The GenElute PCR DNA Purification Kit is for laboratory use only. Not for drug, household or other uses. The Binding Solution contains guanidine HCl which is toxic. Wear gloves, safety glasses, and suitable protective clothing when handling this solution or any reagents provided with the kit. See the Material Safety Data Sheet (MSDS).

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

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 60 ml of 95-100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of ethanol.

3. **1X TE buffer:** If using 100X TE buffer concentrate, dilute 100-fold with molecular biology grade water to prepare 1X TE buffer (10 mM Tris HCl, pH 8, 1 mM EDTA).

Procedure

All steps are carried out at room temperature. Please note that centrifugation speeds are given in units of g. Convert to rpm depending on size of centrifuge rotor, according to 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.
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.

1. Add 0.5 ml of Binding Solution to a microcentrifuge tube containing up to 50 μl of the completed PCR reaction.

2. Place a DNA binding spin column into a 2 ml collection tube; transfer the solution from step 1 onto the column.

3. Centrifuge the column at maximum speed (12,000-16,000 x *g*) for 1 minute. Discard the flow-through eluate, but retain the collection tube.

4. Apply 0.5 ml of diluted Wash Solution to the spin column and centrifuge at maximum speed for 1 minute. Discard flow-through eluate, but retain the collection tube.

5. Centrifuge the column again at maximum speed for 2 minutes, without any additional wash solution, to remove excess ethanol. Discard any residual flow-through as well as the collection tube.

6. Transfer the spin column to a fresh 2 ml collection tube. Apply 50 μl of 1X TE buffer or water to the center of each column. Incubate at room temperature for not more than 1 minute.

7. 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 store 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 (see Preparation Instructions).
	DNA was not eluted properly	DNA must be eluted with a low salt solution such as 1X TE buffer or water.
	Elution solution sat in column too long	Allow the elution solution to sit in the column for not more than one minute.
Performance in downstream enzymatic applications is poor	DNA eluate is contaminated with salt	In step 4 of the Procedure, allow the diluted Wash Solution to sit in the column for 2-5 minutes.
	Eluate is contaminated with ethanol not entirely removed before elution	Be sure to centrifuge at maximum speed in step 5 of Procedure.

Related Products	Product Codes	Related Products	Product Codes
Taq DNA Polymerase	D 1806 D 4545	PCR Core Kit with Taq DNA Polymerase	CORE-T
AccuTaq™LA DNA Polymerase	D 8045	All-in-One Random Prime Labeling Mix, -dCTP	R 7522 R 9647
KlenTaq LA DNA Polymerase Mix	D 5062	All-in-One Random Prime Labeling Mix, -dCTP	R 7022 R 9522
Long PCR Core Kit	LCOR-1	Random Primer DNA Labeling Kit	PRIME-2

‡The PCR process is covered by patents issued to Hoffmann-La Roche Inc.

JHK 1/01

Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.