Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

SeqPure PCR Purification System

Catalog Number: Z1510001, Z1510002, and Z1510003

Introduction

For many molecular biology techniques, removing contaminants from DNA is critical for downstream applications. For example, to ensure a successful sequencing reaction, all excess contaminants such as primers, dNTPs, salts, and residual proteins, must be removed from the template. These agents can interfere with subsequent reactions and lead to unreadable sequences.

Biochain's SeqPure PCR Purification System is designed for rapid and efficient purification of PCR products. The system utilizes paramagnetic bead technology to selectively bind DNA fragments 100 bp and larger in size. Simple washing steps will remove salts, primers, primer-dimers, nucleotides, and enzymes prior to elution of the purified PCR product. The SeqPure DNA Purification System is ideal for purification of DNA using either manual procedures or automated liquid handling instruments.

Features

- Fast – purifies PCR products in under half an hour
- Pure – removes salts, dNTPs, primers, and enzymes
- Simple – no centrifugation or vacuum filtration needed
- Ready-to-use – no fancy buffers; just use ethanol and Tris-HCl /TE
- Economical – same performance as leading competitors at a more budget-friendly cost
- Flexible – process between 1 to 384 samples simultaneously

Applications

- PCR
- Genotyping
- Sequencing
- Cloning
- Microarrays
- Fragment analysis
- Restriction enzyme digestion

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Description
BioChain's SeqPure DNA Purification System is simple and easy: bind DNA to beads, wash out impurities, and elute purified DNA.

1. SeqPure beads are added to the PCR product for DNA binding.

2. During the process, contaminants and salts are washed off.

3. Purified DNA is eluted, ready to be used in subsequent applications.

Quality Control
Each lot has been tested for % recovery rate

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Components
SeqPure DNA Purification System paramagnetic beads solution

Product Number	Description	Number of reactions (for 10 ul PCR reactions	Storage Conditions
Z1510001	SeqPure DNA Purification System - 5 mL	277	2-8° C
Z1510002	SeqPure DNA Purification System - 50 mL	2770	
Z1510003	SeqPure DNA Purification System - 500 mL	27,770	

Reagents and Equipments Required but not Supplied in this Kit:
- 70% Ethanol
- 10mM TRIS-HCL pH:8.0 (DNA elution)
- Reagent grade water
- 1mM EDTA

Magnet (Stand and Plate):
For 96 well format: 96 well ring stand
For 384 well format: 384 Magnet
Reaction Plate:
For 96 well format: 96 Well Cycling Plate
For 384 well format: 384 Well Cycling Plate

Storage and Stability
Store at 2-8° C. This system is stable for one year from date of receipt under proper storage conditions

Protocol

Procedure for 96 Well Format:

1. Determine whether or not it is necessary to transfer the samples to a reaction plate.
Note: If PCR reaction volume is >20 µL a plate transfer is required.

2. Gently shake the SeqPure bottle to resuspend any beads that may have settled. Add 1.8 ul of SeqPure beads per 1.0 ul of PCR product to each well (see table below).

PCR Reaction Volume (µL)	SeqPure Volume (µL)
10	18
20	36
50	90

Use the following equation to determine the volume of SeqPure beads per PCR reaction:
SeqPure volume per reaction= 1.8 x (PCR Reaction Volume)

3. Mix SeqPure and PCR reaction thoroughly by pipetting up and down 6-8 times.

4. Incubate the mixture for 5 minutes at room temperature for maximum recovery

5. Place the reaction plate on a magnetic separation device for 2-3 minutes.
IMPORTANT: wait until the solution is clear before proceeding; otherwise there may be bead loss which will lower the subsequent yield
Note: Bead separation is dependent on the quality of the magnetic separation device.

6. With the plate still on the magnet, aspirate and discard supernatant.
Note: Do not disturb the beads while aspirating the supernatant. Remove supernatant from the opposite side of the well.

7. With the plate still on the magnet, add 200µL of 70% Ethanol to each well of the reaction plate. Incubate for 30 secs at room temperature. Aspirate and discard the ethanol**.**
Note: Remove as much of the Ethanol as possible.

8. Repeat step 7 for a total of 2 ethanol washes.

9. After aspirating the ethanol leave the plate on the magnetic separation device for 3-5 min at room temperature to evaporate the remaining traces of alcohol*.*
Note: Do not over dry the beads as this will significantly decrease yield.

10. Remove the reaction plate from the magnetic separation device.

11. Add 40µL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE buffer) to each well of the reaction plate and mix by pipeting up and down 10 times.
Note: Pre-warming elution buffer at 55°C can increase the yield.

12. Incubate at room temperature for 2-3 minutes.

13. Place the reaction plate onto the magnetic separation device for 2-3 minutes or until the magnetic beads separate from the solution.

14. Transfer the eluate to a new plate for analysis. For storage, seal with non-permeable sealing film. For short-term storage (a few days), samples should be kept at 2-8°C. For long-term storage, samples should be kept at -20°C.

Procedure for 384 Well Format:

1. Transfer the sample to an appropriate 384-well plate.

2. Gently shake the SeqPure bottle to resuspend any beads that may have settled. Add 1.8 ul of SeqPure beads per 1.0 ul of PCR product to each well (see table below).

PCR Reaction Volume (µL)	SeqPure Volume (µL)
5	9
7	12.6
10	18

Use the following equation to determine the volume of SeqPure beads per PCR reaction:
SeqPure volume per reaction= 1.8 x (PCR Reaction Volume)

3. Mix SeqPure and PCR reaction thoroughly by pipetting up and down 6-8 times.

4. Incubate the mixture for 5 minutes at room temperature for maximum recovery.

5. Place the reaction plate on magnetic separation device for 2-3 minutes for separation **IMPORTANT**: wait until the solution is clear before proceeding otherwise there may be bead loss which will lower the subsequent yield
Note: Bead separation is dependent on the quality of the magnetic separation device.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

6. With the plate still on the magnet, aspirate and discard supernatant.
   Note: Do not disturb the beads while aspirating the supernatant. Remove supernatant from the opposite side of the well.

7. With the plate still on the magnet, add 30µL of 70% Ethanol to each well of the reaction plate. Incubate for 1 minute at room temperature. Aspirate and discard the ethanol**.**
   Note: Remove as much of the Ethanol as possible.

8. Repeat step 7 for a total of 2 ethanol washes.

9. After aspirating the ethanol leave the plate on the magnetic separation device for 3-5 min at room temperature to evaporate the remaining traces of alcohol
   Note: Do not over dry the beads as this will significantly decrease yield.

10. Remove the reaction plate from the magnetic separation device.

11. Add 30 µL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE buffer) to each well of the reaction plate and mix by pipeting up and down 5 times.
    Note: Pre-warming elution buffer at 55°C can increase the yield.

12. Incubate at room temperature for 2-3 minutes.

13. Place the reaction plate onto the magnetic separation device for 2 minute or until the magnetic beads separate from the solution.

14. Transfer the eluate to a new plate for analysis. For storage, seal with non-permeable sealing film. For short-term storage (few days), samples should be kept at 2-8°C. For long-term storage, samples should be kept at -20°C.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Size Selection Protocol for SeqPure

Catalog Number: Z1510001, Z1510002, and Z1510003

Supplemental Instruction:
Please read the full user's manual for SeqPure PCR Purification System before using this supplemental protocol. This is an abbreviated protocol specifically for size selection.

Recommended Conditions for Size Selection:

Approximate Insert Size	300 bp	330bp	550 bp	700bp
Bead: DNA ratio				
1st bead selection	0.75x	0.7x	0.55x	0.5x
2nd bead selection	0.1x	0.1x	0.1x	0.1x

Recommended Protocol for size selection:

Example: selection for 550bp

Bead selection to remove larger fragments:
1. Add 50 µl of 10 ng/µl sheared genomic DNA to a 96 well plate. Add 27.5 µl (0.55X) of resuspended SeqPure beads. Mix well by pipetting.
2. Incubate for 3 minutes at room temperature.
3. Place the plate on the magnetic stand for 2 minutes (or until clear) to separate the beads from supernatant.
4. Transfer supernatant to a new plate. **DO NOT** discard the **supernatant**.
5. Discard beads that contain the large fragments. Continue with step 6.

Bead selection to removed smaller fragments:
6. Add 5 µl resuspended SeqPure beads (0.1X) to the supernatant from step 4 . Mix well by pipetting up and down for 10 times.
7. Incubate for 3 minutes at room temperature.
8. Place the plate on the magnetic stand for 2 minutes (or until clear) to separate the beads from supernatant.
9. Remove and discard supernatant. **DO NOT** discard the **beads**.
10. Add 200 µL 80% freshly prepared ethanol while plate is on the magnet. Incubate for 30 seconds at room temperature. Remove supernatant.
11. Repeat step 10 once .
12. Air dry the beads on the magnetic stand for 7 minutes. *Note: Do not over dry the beads as this will decrease the recovery of target DNA.*
13. Remove the plate from the magnet. Elute DNA with 25 µL elution buffer and mix well by pipetting up and down for 10 times. Incubate for 2 minutes at room temperature.
14. Place the plate on the magnetic stand until the solution is clear, approximately 1 minute. Transfer the eluate to a new plate.