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PCR and Sequencing Reaction Clean-Up Kit (Magnetic Bead System) – 50 preps

Product #60200 **Product Insert**

Norgen's PCR and Sequencing Reaction Clean-Up Kit (Magnetic Bead System) provides a rapid, simple and efficient procedure for the purification and clean-up of amplified DNA products from PCR mixes, as well as sequencing and various other enzymatic reactions including restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations. This kit is able to effectively remove PCR by-products including primers, dimers, enzymes, unincorporated nucleotides and mineral oil from the desired PCR product. In addition, the kit is used to remove reaction contaminants including dye terminators, salts, enzymes, excess primers and primer dimers. Contaminants are undesirable as they can interfere with many downstream applications including sequencing, PCR, RFLP, restriction enzyme digestions and ligation. Purification is based on a magnetic bead system, which replaces centrifuge steps and requires a minimal laboratory equipment. The kit provides a high quality product with up to 90% recovery.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized binding conditions. The process involves first mixing the sample containing the enzymatic reaction or PCR with Binding Buffer C (please see the flow chart on page 3). The Magnetic Bead Suspension is then added to the mixture and the resulting solution is placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, and the bound DNA is then washed with 70% ethanol in order to remove any remaining impurities. The purified total DNA is then eluted with the Elution Buffer B. The purified product is of the highest integrity, and can be used in sequence analysis and other downstream applications.

Kit Components:

Component	Product # 60200 (50 samples)
Binding Buffer C	30 mL
Elution Buffer B	8 mL
Magnetic Bead Suspension	1.1 mL
Elution tubes (1.7 mL)	50
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Advantages:

- **Purification from all PCR by-products** – Remove primers, dimers, enzymes, unincorporated nucleotides and mineral oil from the desired PCR product
- **Purification from all sequence cycling by-products** - Remove all dye terminators, primer-dimers, primers, PCR enzymes and salts.
- **Purification of all types of enzymatic reactions** - Purify DNA from different enzymatic reactions including PCR, restriction enzyme digests, Klenow reactions, alkaline phosphatase reactions, and ligations.
- **High recovery** - Recovery of up to 90% of input amount.
- **Complete magnetic bead purification** – The PCR product or enzymatic reaction is magnetic bead cleaned without the use of phenol, chloroform or alcohol precipitation.
- **High integrity product** – The cleaned extension product or PCR reaction is ready to be used in various downstream applications.

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Binding Buffer C contains guanidinium salts, and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- Nuclease-free water
- 96 - 100% ethanol
- Magnetic bead separation rack
- 70% ethanol (prepare fresh)
- 65ºC incubator

Procedures

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.
- **Always vortex** the **Magnetic Bead Suspension** before use.
- Preheat an incubator to 65ºC

Flow Chart

Procedure for the Clean-Up of PCR and Sequencing Extension Products and Enzymatic Reactions

Add 5 volumes of Binding Buffer C to the extension product or ezymatic reaction. Mix well.

↓

Add ethanol Magnetic Bead Suspension. Mix. Incubate for 5 minutes.

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Place tube in magnetic separation rack. Let stand for 1 minute.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute. ↓

Place tube in magnetic separation rack. Let stand for 1 minute.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute. ↓

Incubate open tube at 65˚C for 5 minutes.

Add Elution Buffer B, mix and Incubate at 65˚C for 10 minutes. ↓

Place tube in magnetic separation rack. Let stand for 1 minute.

Carefully transfer supernatant to Elution Tube. ↓

Purified Extension Product or Enzymatic Reaction Product

1. Sample Preparation

a. Add 5 volumes of **Binding Buffer C** directly to the tube containing the extension product or PCR reaction and mix well. Vortex and pulse-spin briefly in microcentrifuge to aid in mixing.
b. Add 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the mixture above.
c. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
d. Proceed to Section 2: DNA Isolation.

2. DNA Isolation

a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute.
b. Aspirate and discard supernatant without touching the magnetic beads.
c. Remove the sample tube from the magnetic rack and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
d. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
e. Aspirate and discard supernatant without touching the magnetic beads.
f. Repeat **Steps 2c – 2e** for a second wash step.

Note: Remove as much of the 70% ethanol in the sample tube as possible by pipetting.

g. Incubate the open tube at 65˚C for 5 minutes to dry the magnetic beads.
h. Remove the sample tube from the magnetic rack and add 50 µL of **Elution Buffer B**. Mix by vortexing and incubate at 65˚C for 10 minutes.
i. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
j. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage

Troubleshooting Guide

Problem	Possible cause	Solution and Explanation
Poor DNA recovery	Binding of DNA to the Magnetic beads was inefficient	Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of **Binding Buffer C** was used for the volume of the extension product.
	Binding Buffer C was not completely removed in the wash step.	Traces of salt left on the magnetic beads from the binding step may interfere with the elution of the DNA. Ensure that the magnetic beads were washed twice with the 70% Ethanol (freshly prepared).
	Proper Elution Buffer was not used	The provided **Elution Buffer B** has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8.
DNA does not perform well in downstream applications	Insufficient washing of magnetic beads and ethanol carry over	Traces of salt from the binding step may remain in the sample if the magnetic bead is not properly washed twice with the **70% Ethanol** (freshly prepared). Also ensure that the magnetic bead is dried at 65ºC for 5 minutes to prevent ethanol carry over.

Related Products	Product #
Sequencing Reaction Clean-Up 96-Well Kit	34400
PCR Purification Kit	14400
DNA Gel Extraction Kit	13100

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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