User Guide

NuPure PCR Cleanup Kit

For purification of PCR products (100 bp – 10 kb)

Version **190422**

Catalog **K0321-SMP**

K0321-100

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Product description

The NuPure PCR Cleanup Kit is designed to eliminate primers, unincorporated dNTPs, enzymes, and other impurities from PCR products for downstream applications such as sequencing, PCR, mapping, and cloning. The unique magnetic beads used in this kit feature a high binding capacity, slow sedimentation rate and have been designed to selectively bind PCR amplicons 100 bp and larger without salt carryover.

Contents and storage

Reagents have a one (1) year expiration date from date of purchase when stored properly.

NuPure PCR Cleanup Catalog no.	4 preps [3] K0321-SMP	100 preps [3] K0321-100	Storage
NuPure Beads [1]	500 ul	10 ml	2-8°C
Wash Buffer [1] *Customer adds Ethanol*	400 ul	8 ml	15-30°C
Elution Buffer [1]	500 ul	10 ml	15-30°C

Note. Some reagents in each kit are provided more than the amount required.

[1] NuPure Beads are also available as Catalog no. A1419 and Wash Buffer is available as Catalog no. R2300 and Elution Buffer is available as R0510.

[2] Prior to first time use, add the required amount of alcohol as indicated on the label of each bottle, then check the box and mix well.

[3] The number of preps or isolations are based on 50 ul PCR product per prep.

Required materials not supplied

Item	Source	Catalog no.
Ethanol, absolute (100%)	Fisher Scientific	BP291914
Micro- and conical tubes (1-15 ml)	Various	Not applicable
Magnetic separator for micro-tubes	Ocean NanoTech	MMS
Vortex mixer	Various	Not applicable
Pipettors (20-1000 ul) and tips	Various	Not applicable

Principle

The magnetic bead technology in this kit enables ratio-metric control of DNA fragment size isolation for downstream processing. By adjusting the volume of NuPure Beads to sample volume, one can tune the size distribution of isolated nucleic acids. After a quick binding reaction, beads are washed, air-dried and nucleic acids are eluted in a user-defined volume.

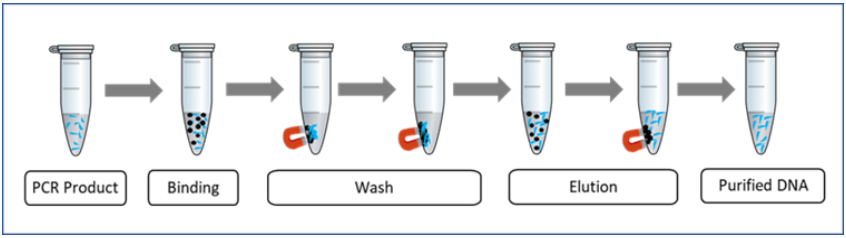

Figure. Illustration showing magnetic separation of DNA fragments using the NuPure PCR Cleanup Kit.

Procedural guidelines

- Read the USER GUIDE and make sure all the directions are followed and all recommended volumes are used as indicated.
- Prior to use, bring all kit components to room temperature.
- All vortex steps should be performed at maximum speed to ensure mixing.
- NuPure Beads should be resuspended to a homogenous suspension prior to use.

Before you begin

Prepare working buffers

Prior to first time use, add ethanol (100%) as indicated on the label of each bottle(s) of Wash Buffer to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

Protocol: Cleanup PCR products or DNA sample

The following protocol is for cleaning up 50 µl PCR products or DNA sample. This can be scaled up or down depending on input and elution volumes up to a maximum of 100 µl. If the amount of PCR product or DNA sample is less than or greater than 50 µl, proportionally increase the volumes of NuPure Beads.

Bind PCR products to beads

1. Add 90 ul NuPure Beads and 50 ul PCR product to a micro tube.
2. Pulse vortex the sample or pipette up and down 10 times to bind the DNA to the beads. Then incubate the mixture at room temperature for 5 minutes.
3. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

Wash NuPure Beads with wash buffers

4. Keep tubes on the magnetic separator. Add 200 ul of Wash Buffer and incubate for 30 seconds. Then discard all the supernatant.
5. Repeat Steps 4 for a second wash with Wash Buffer.
6. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 5 minutes. Remove any visible supernatant without disturbing the beads.

   Caution. Do not over dry beads as it may result in loss of cfDNA.

Elute the cfDNA

7. Remove the tube from magnetic separator and add 100 ul of Elution Buffer. Completely resuspend beads by pipetting up and down 10 times. Then incubate for 2 minutes at room temperature.
8. Place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.
9. Carefully transfer the supernatant containing the purified genomic DNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

   Note. The purified DNA is ready for immediate use. Alternatively, you can store the DNA at 4˚C for up to 24 hours or -20˚C for long-term storage.

Yield and quality measurements

DNA yield

We recommend using the Invitrogen™ Qubit™ dsDNA BR Assay Kit (Catalog no. Q32850 for total genomic DNA yield and quantification. This assay is specifically designed for use with the Qubit Fluorometer but can be used with any fluorometer or fluorescence plate reader.

DNA quality

We recommend using the NanoDrop or any spectrophotometer to determine the DNA quality. The NanoDrop is able to measure a sample using just 1 ul of sample and in less than five seconds to assess the purity of the sample such as proteins and nucleic acids.

Document History

Version	Person	Description of Change
190702	Luis Moreno	Initial draft
180716	Luis Moreno	Consultation draft – to Alice Bu
190422	Luis Moreno	Final version – approved by Luis Moreno

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Warranties and Disclaimers

The Ocean NanoTech products ("Product") is warranted to operate or perform in conformance with published Product specifications at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by professionally trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper, and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, expressed or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any purpose, or non-infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of accident, disaster or event of force majeure, misuse, fault or negligence of or by Buyer, or use of the Products in a manner for which they were not designed, or improper storage and handling of the Products.