Instructions For Use

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AmpliClean™

Cleanup Kit, Magnetic Beads

For PCR Purification, NGS Library Cleanup and Size Selection

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

AmpliClean™ Cleanup Kit, Magnetic Beads

REF AP-005, AP-050, AP-500

Research Use Only

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Symbols Used on Product Labels and in Instructions For Use

Symbol	Description
	Manufacturer
	Use-by date
	Lot number
	Reference number
	Temperature limit for storage
	Contains sufficient for <*n*> tests
	Matrix code containing the reference number, lot number and use-by date

Product Description

The AmpliClean™ Cleanup Kit delivers high recovery, superior quality purified DNA without salt carryover. It utilizes Solid Phase Reversible Immobilization (SPRI) paramagnetic bead-based technology for low- to high-throughput DNA purification in a variety of applications including PCR, Next-generation Sequencing (NGS) library preparation and microarray. AmpliClean™ is the widely adopted equivalent for the gold standard (AMPure XP) for consistent size selection and cleanup within NGS workflows.

The AmpliClean™ workflow involves three simple steps: bind, wash and elute. While binding the PCR product selectively to the magnetic beads, unincorporated dyes, nucleotides, salts and primers will be effectively removed during an ethanol wash, leaving ultra-pure DNA of the desired length.

The workflow does not involve any centrifugation or vacuum filtration steps and is therefore amendable for full automation using liquid handlers, in conjunction with Alpaqua® 96-well or 384-well Magnet Plates. It can also easily be performed manually.

Kit Contents and Storage

AmpliClean™ Cleanup Kits include a ready-for-use magnetic bead solution for purification of PCR products in a 96-well or 384-well plate, or 1.5 mL Eppendorf tube (for pooled samples):

Reference	Volume	# Reactions (10 µL, 96-well)	# Reactions (5 µL, 384-well)	# Reactions (100 µL, 1.5 mL tube)	Storage
AP-005	5 mL	278	556	28 pools	Store kit at 4 °C, protected from light. Do not freeze.
AP-050	50 mL	2780	5560	280 pools	
AP-500	500 mL	27800	55600	2800 pools	

Required Materials, Not Included

Description
Ethanol 70%, molecular biology grade
Elution Buffer (0.1 mM EDTA pH 8.0, or diH_2O)
96- or 384-well PCR plates
1.5 ml Eppendorf tubes
(Multichannel) Pipettes, including disposable filter tips
Alpaqua® Magnet Plate, 96-well or 384-well
Magnet Stand, for 1.5 mL Eppendorf tube

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice and wear protective eyewear, gloves and lab coat when handling the magnetic bead suspension supplied in this kit. Wash body parts with ample amount of water immediately if they come in contact with the bead suspension. Seek medical help if needed.

Protocol (96-well)

1. Resuspend the AmpliClean™ bead solution by gently shaking.

2. Add 1.8 µL of AmpliClean™ per 1.0 µL of PCR product to each PCR well in the 96-well reaction plate. Add bead solution according to the sample reaction volume shown:

Sample Volume (µL)	AmpliClean™ Volume (µL)
10	18
20	36
50	90
100	180

3. Mix beads and sample thoroughly by pipette mixing 10 times and incubate for 3 - 5 minutes at room temperature.

4. Place the reaction plate onto a 96-well magnet plate (e.g. Alpaqua® 96 Super Magnet Plate or Alpaqua® MAGNUM FLX® Enhanced Universal Magnet Plate) for 2 minutes, to separate beads from the solution.

   NOTE: Wait for the solution to clear before proceeding to the next step.

5. While sitting on the magnet plate, aspirate the cleared solution from the reaction plate and discard by pipetting from the center of the bottom of the wells.

   NOTE: Make sure the removed solution is fully cleared and not to disturb the ring of separated magnetic beads on the side of the well.

6. While leaving the plate on the magnet, immediately dispense 150 µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature.

7. Remove the ethanol and discard. Repeat this step and make sure to completely remove all ethanol with the last aspiration.

   OPTION: Dry for a maximum of 5 minutes at room temperature. Do not over-dry.

8. Remove the reaction plate from the magnet and add 40 µL of elution buffer to each well of the reaction plate and homogenize the beads in the elution buffer by pipette mixing 10 times. Incubate for 2 minutes.

9. Place the reaction plate onto the magnet plate for 1 minute to separate beads from the solution.

10. Transfer the eluant, containing the purified PCR products, to a new 96-well plate.

    NOTE: 5 – 10 µL of cleared solution can be left behind in the original reaction plate to prevent bead transfer, as it can interfere with injection. If beads do transfer, place the samples back onto the original reaction plate and re-transfer onto a new reaction plate.

Protocol (384-well)

1. Resuspend the AmpliClean™ bead solution by gently shaking.

2. Add 1.8 µL of AmpliClean™ per 1.0 µL of PCR product to each PCR well in the 384-well reaction plate. Add bead solution according to the sample reaction volume shown:

Sample Volume (µL)	AmpliClean™ Volume (µL)
5	9
7	12.6
10	18
14	25

3. Mix beads and sample thoroughly by pipette mixing 10 times and incubate for 3 - 5 minutes at room temperature.

4. Place the reaction plate onto a 384-well magnet plate (e.g. Alpaqua® 384 Post Magnet Plate) for 2 minutes, to separate beads from the solution.

   NOTE: Wait for the solution to clear before proceeding to the next step.

5. While sitting on the magnet plate, aspirate the cleared solution from the reaction plate and discard by pipetting from the center of the bottom of the wells.

   NOTE: Make sure the removed solution is fully cleared and not to disturb the ring of separated magnetic beads on the side of the well.

6. While leaving the plate on the magnet, immediately dispense 30 µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature.

7. Remove the ethanol and discard. Repeat steps 6 and 7 and make sure to completely remove all ethanol with the last aspiration.

   OPTION: Dry for a maximum of 5 minutes at room temperature. Do not over-dry.

8. Remove the reaction plate from the magnet and add 30 µL of elution buffer to each well of the reaction plate and homogenize the beads in the elution buffer by pipette mixing 10 times. Incubate for 2 minutes.

9. Place the reaction plate onto the magnet plate for 1 minute to separate beads from the solution.

10. Transfer the eluant, containing the purified PCR products, to a new 384-well plate.

    NOTE: 2 – 5 µL of cleared solution can be left behind in the original reaction plate to prevent bead transfer, as it can interfere with injection. If beads do transfer, place the samples back onto the original reaction plate and re-transfer onto a new reaction plate.

Protocol (1.5 mL tube, pooled sample)

1. Resuspend the AmpliClean™ bead solution by gently shaking.

2. Mix 100 µL beads with 100 µL pooled sample thoroughly in a 1.5 mL Eppendorf reaction tube by pipette mixing 5 times. Incubate for 5 minutes, off-magnet, at room temperature.

   NOTE: The volume of AmpliClean™ beads should be adjusted so that an equal ratio (1x) of NGS library pool to AmpliClean™ beads is achieved. Increased magnet incubation times may be necessary for larger volumes.

3. Place the reaction plate onto a magnet stand designed for 1.5 mL Eppendorf reaction tubes for 3 minutes, to separate beads from the solution.

   NOTE: Wait for the solution to clear before proceeding to the next step.

4. While sitting in the magnet stand, aspirate the cleared solution from the reaction tube and discard by pipetting from the center of the bottom of the wells.

   NOTE: Make sure the removed solution is fully cleared and not to disturb the ring of separated magnetic beads on the side of the well.

5. While leaving the tube in the magnet stand, immediately dispense 200 µL of 70% ethanol into the reaction tube without disturbing the beads and incubate for 1 minute at room temperature.

6. Remove the ethanol and discard. Repeat steps 5 and 6 and make sure to completely remove all ethanol with the last aspiration.

7. Dry for a maximum of 3 minutes at room temperature. Do not over-dry.

8. While leaving the tube in the magnet stand, add 100 µL of elution buffer to the tube.

9. Remove the tube from the magnet stand and homogenize the beads in the elution buffer by pipette mixing 10 times, or short vortexing. Incubate for 2 minutes.

10. Place the tube onto the magnet stand for 1- 3 minutes to separate beads from the solution.

    NOTE: Wait for the solution to clear before proceeding to the next step.

11. Transfer 90 µLof clear eluant, containing the purified PCR products, to a new 1.5 mL Eppendorf tube.

    NOTE: 10 µL of cleared solution can be left behind in the original reaction tube to prevent bead transfer, as it can interfere with injection. If beads do transfer, place the samples back into the original reaction tube and re-transfer into a new reaction tube.

Size Selection

NGS relies on having high quality libraries. Part of this is making sure library fragment sizes are within the optimum range for a given sequencing instrument, typically 200-500 bp for Illumina systems.

AmpliClean™ magnetic beads are designed to purify DNA fragments of a desired size for use in downstream applications such as NGS. Following magnetic bead-based cleanup, the final concentration of the libraries can be determined by a double Qubit™ (HS) measurement, according to manufacturer's manual. Size, quantity and integrity of the libraries are typically verified on Agilent TapeStation or *Agilent* Bioanalyzer, according to the manufacturer's protocol.

Customer Support

For technical assistance, please contact us at techsupport@nimagen.com.

Revision History

Section	Summary of changes	Version	Date
All	Not applicable. New document.	1.0	2013-09-01
All	New layout. New introduction (Product Description). Kit Contents and Storage. General Precautions. Protocol for 384-well plate and 1.5 mL Eppendorf tube.	2.0	2023-06-28

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