# Instructions For Use

*Version: 2.0* *Ref: IFU-AMPLIC* *Revision date: 2024-02-13*

# 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 | Lot number |
| REF | Reference number |
| | Temperature limit for storage |
| Σ | 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 paramagnetic bead-based technology for low- to high-throughput DNA purification in a variety of applications including PCR, Next-generation Sequencing (NGS) library preparation. AmpliClean™ is the widely adopted equivalent for the gold standard 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 (i.e. Alpaqua®) 96-well or 384-well Magnet Plates. It can also easily be performed manually on single-tube magnet stands.

## Kit Contents and Storage

AmpliClean™ Cleanup Kits include a ready-for-use magnetic bead solution.

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

Volumes are based on standard purification protocol (1.8x bead volume). For size selection bead ratios, please refer to the respective IFU of your NGS library prep kit.

## Required Materials, Not Included

| Description |
|---|
| Ethanol 70%, molecular biology grade |
| Elution Buffer (0.1 mM EDTA pH 8.0, or di$H_2O$) |
| 96- or 384-well PCR plates or single tubes |
| (Multichannel) Pipettes, including disposable filter tips |
| (Alpaqua®) Magnet Plate, 96-well or 384-well or single tubes |

## 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) for standard purification

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

   NOTE: during pipetting, always make sure to keep the beads in homogeneous suspension.

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 immediately and thoroughly by pipetting up and down 5 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 step 6 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 pipetting up and down 5 times. Incubate for 2 minutes.

9. Place the reaction plate onto the magnet plate for 2 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) for standard purification

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

   NOTE: during pipetting, always make sure to keep the beads in homogeneous suspension.

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 pipetting up and down 5 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 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 pipetting up and down 5 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.

## Size Selection

AmpliClean™ magnetic beads can be used to purify DNA fragments of a desired size for use in downstream applications such as NGS. Following magnetic bead-based cleanup and/or size selection, the final concentration of the libraries can be determined by various methods. Size, quantity and integrity of the libraries are typically verified with gel or capillary electrophoresis. For size selection protocols using AmpliClean, please refer to the respective IFU of your NGS library prep kit. Use the same recommended ratios as the gold standard magnetic bead cleanup kit.

## 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 size selection process. | 2.0 | 2024-02-13 |

## Legal Notice



## Disclaimer



## Published by

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**Manufacture of molecular biology reagents and kits for research use only.**

For and on behalf of BSI:

Matt Page, Managing Director Assurance - UK & Ireland

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For and on behalf of BSI:

Graeme Tunbridge, Senior Vice President Medical Devices

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