# Instructions For Use

*Version: 2.1* *Ref: IFU-DPURE* *Revision date: 2023-06-23*

# D-Pure™

## Dye Terminator Removal Kit

For BrilliantDye™ and BigDye® v1.1 and v3.1 Chemistries

NimaGen.

Innovators in DNA Sequencing Technologies

## Product and Company Information

**D-Pure™ Dye Terminator Removal Kit**

REF DP-005, DP-050, DP-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 D-Pure™ Dye Terminator Removal Kit, based on magnetic bead technology, effectively purifies Dye Terminator Cycle Sequencing reactions. The D-Pure™ workflow involves three simple steps: bind, wash and elute. While binding the sequencing product selectively to the magnetic beads, unincorporated dyes, nucleotides, salts and primers will be removed during ethanol washes. This allows for elution of the pure Sanger sequencing product in the elution buffer of choice.

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.

D-Pure™ is compatible with both NimaGen BrilliantDye™ and Thermo Fisher BigDye® Terminator Cycle Sequencing Kits (v1.1 and v3.1). D-Pure™ is widely adopted as a proven, high-quality purification reagent for laboratories using 3130, 3500, 3730 or SeqStudio™ Series Genetic Analyzers. Purified dye-labeled extension products can be loaded directly on the Genetic Analyzer without the need for resuspension.

## Kit Contents and Storage

D-Pure™ Dye Terminator Removal Kits include a ready-for-use magnetic bead solution for purification of 500 up to 100000 cycle sequencing reactions, using a 96-well or 384-well plate format:

| Reference | Volume | # Reactions (96-well) | # Reactions (384-well) | Storage |
|---|---|---|---|---|
| DP-005 | 5 mL | 500 | 1000 | Store kit at 4 °C, protected from light |
| DP-050 | 50 mL | 5000 | 10000 | |
| DP-500 | 500 mL | 50000 | 100000 | |

## Required Materials, Not Included

| Description |
|---|
| Ethanol 80%, molecular biology grade |
| Elution Buffer (0.1 mM EDTA pH 8.0, or di$H_2O$) |
| 96- or 384-well plates, compatible with Genetic Analyzer |
| (Multichannel) Pipettes, including disposable filter tips |
| Alpaqua® Magnet Plate, 96-well or 384-well |

## 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 D-Pure™ bead solution by shaking.
2. Add 10 µL of homogenized D-Pure™ bead solution into each sample.
3. Add 42 µL (for 10 µL sequencing reactions) or 62 µL (for 20 µL sequencing reactions) of 75% ethanol into each sample and immediately mix by pipetting up and down.
4. Place the sample plate onto the 96-well magnet plate; wait 3 min or until the solution is clear.
5. While the plate is on the magnet, aspirate the solution (supernatant) from the sample wells and discard. Ensure not to disturb the beads; avoid pipetting from the bottom of the wells.
6. While on magnet, add 100 µL of 75% ethanol into each well; wait 30 sec.
7. While on magnet, aspirate the ethanol and discard.
8. Repeat steps 6 and 7 for a total of two ethanol washes. Especially for the last aspiration step, ensure removing the ethanol completely.
9. Off magnet, air-dry the sample at room temperature for 3 - 10 min. Do not over dry, as it can degrade the fluorescent dye.
10. Add 40 µL of elution buffer (0.1 mM EDTA pH 8.0 or di$H_2O$), mix and incubate at room temperature for 5 min.
11. Place the sample plate on the magnetic plate; wait 3 min or until the solution is clear.
12. While keeping the sample plate on the magnet, transfer 30 - 35 µL of cleared solution into a new plate, compatible with the Genetic Analyzer. The samples are now ready for injection.

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

## Protocol (384-well)

1. Resuspend the D-Pure™ bead solution by shaking.
2. Add 5 µL of homogenized D-Pure™ bead solution into each sample.
3. Add 31 µL (for 5 µL sequencing reactions) of 75% ethanol into each sample and immediately mix by pipetting up and down.
4. Place the sample plate onto the 384-well magnet plate; wait 3 min or until the solution is clear.
5. While the plate is on the magnet, aspirate the solution (supernatant) from the sample wells and discard. Ensure not to disturb the beads; avoid pipetting from the bottom of the wells.
6. While on magnet, add 40 µL of 75% ethanol into each well; wait 30 sec.
7. While on magnet, aspirate the ethanol and discard.
8. Repeat steps 6 and 7 for a total of two ethanol washes. Especially for the last aspiration step, ensure removing the ethanol completely.
9. Off magnet, air-dry the samples at room temperature for 10 min. Do not over dry, as it can degrade the fluorescent dye.
10. Add 25 µL of elution buffer (0.1 mM EDTA pH 8.0 or di$H_2O$), mix and incubate at room temperature for 5 min.
11. Place the sample plate on the magnetic plate; wait 3 min or until the solution is clear.
12. While keeping the sample plate on the magnet, transfer 20 µL of cleared solution into a new plate, compatible with the Genetic Analyzer. The samples are now ready for injection.

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

## 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. | 2.0 | 2018-01-24 |
| All | New layout. New introduction (Product Description). Kit Contents and Storage. General Precautions. | 2.1 | 2023-06-23 |

## Legal Notice



## Disclaimer



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