Instructions For Use

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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

RUO Research Use Only

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bsi QUALITY MANAGEMENT SYSTEM
ISO 9001:2015 FM 711484
ISO 13485:2016 MD 711483

Symbols Used on Product Labels

Symbol	Description
	Manufacturer
	Use-by date
	Lot number
	Reference number
	Research Use Only
	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 310, 3100, 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 100.000 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	1.000	Store kit at 4°C, protected from light
DP-050	50 mL	5.000	10.000	
DP-500	500 mL	50.000	100.000	

Required Materials, Not Included

Description
Ethanol 80%, molecular biology grade
Elution Buffer (0.1 mM EDTA pH 8.0, or diH_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™ beads solution by shaking
2. Add 10 µL 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 80% 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 by pipetting from the bottom of the wells.
6. While on magnet, add 100 µL of 80% 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 sample at room temperature (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 diH2O), mix and incubate at room temperature (5 min).
11. Place sample plate on magnetic plate; wait (3 min, or until solution clears).
12. While keeping 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 µL – 10 µL of cleared solution is left behind to prevent bead transfer, as it can interfere with injection. If beads do transfer, place samples back onto original plate and re-transfer onto a new plate.

Protocol (384-well)

1. Resuspend the D-Pure™ beads solution by shaking
2. Add 5 µL homogenized D-Pure™ bead solution into each sample.
3. Add 31 µL (for 5 µL sequencing reactions) of 80% 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 by pipetting from the bottom of the wells.
6. While on magnet, add 40 µL of 80% 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 sample at room temperature (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 diH_2O), mix and incubate at room temperature (5 min).
11. Place sample plate on magnetic plate; wait (3 min, or until solution clears).
12. While keeping 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 samples back onto original plate and re-transfer onto a new plate.

Customer Support

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

Legal Notice

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