D-Pure™ Dye Terminator Removal Kit

Quick Reference Guide

Version: 2.0
Revision date: 24-01-2018

Product and Company Information

Product name: D-Pure™ DyeTerminator Removal kit

Product use: For Research Use Only

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Description

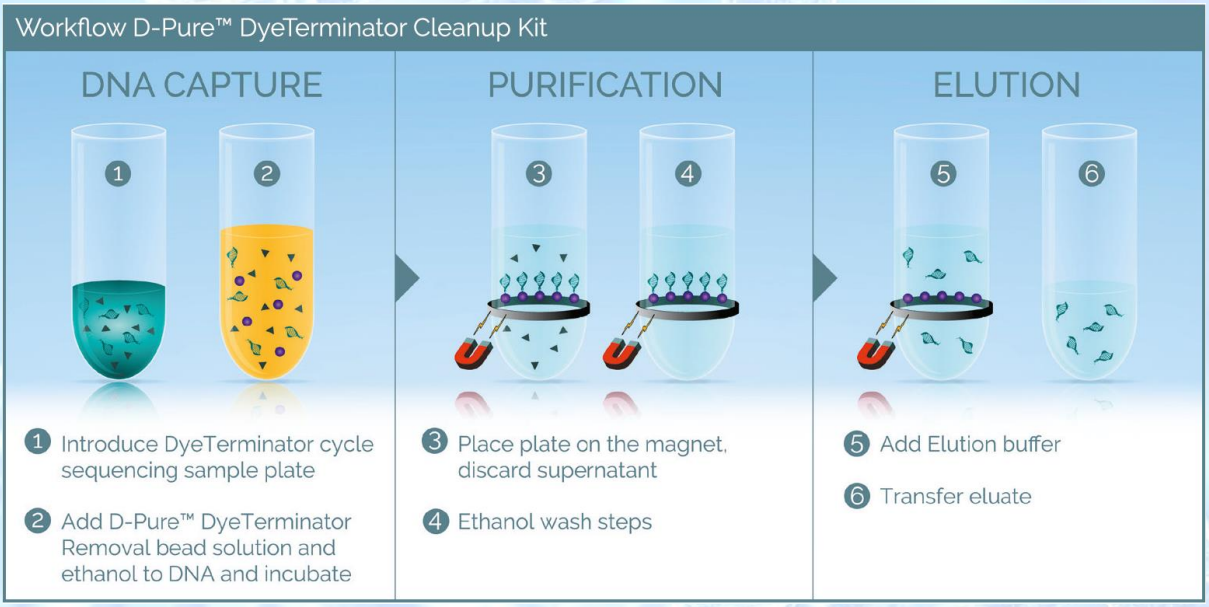
The D-Pure™ Dye Terminator Removal kit, based on magnetic bead technology, purifies Dye Terminator Cycle Sequencing reactions, by capturing the sequencing extension products and removing unincorporated dyes, nucleotides and salts. The workflow does not involve any centrifugation or vacuum filtration steps, and is therefore amendable for full automation using liquid handlers. D-Pure™ is compatible with BrilliantDye™ Terminator Cycle Sequencing kits, v1.1 and v3.1 (NimaGen) and with BigDye® Terminator Cycle Sequencing kits, v1.1 and v3.1 (ThermoFisher Scientific).

Kit Content

p/n	content	# Reactions (96 well format)	# Reactions (384 well format)
DP-005	5 mL	500	1000
DP-050	50 mL	5,000	10,000
DP-500	500 mL	50,000	100,000

Needed but not included

- Ethanol (molecular biology grade), 80%
- Elution Buffer (0.1 mM EDTA pH 8.0 or DiH2O)
- (Multichannel) Pipettes, including disposable tips
- 96 or 384 well plates, compatible with Genetic Analyzer
- Magnet Plate, 96 or 384 well format (Alpaqua, available thru NimaGen)



Protocol (96 well format)

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 ring magnet plate and wait for 3 minutes or until the solution is clear.
5. While the plate is on the magnet, aspirate the solution (supernatant) from the sample wells and discard. Make sure 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 and wait 30 seconds.
7. While on magnet, aspirate ethanol and discard.
8. Repeat steps 6 and 7 for a total of two ethanol washes. Especially for the last aspiration step, make sure to remove the alcohol completely.
9. Off magnet, air-dry sample at room temperature for 10 minutes. Do not over dry.
10. Add 40 µl of elution buffer (0.1 mM EDTA pH 8.0 or DiH_2O), mix, and incubate at room temperature for 5 minutes.
11. Place sample plate on magnetic plate and wait 3 minutes 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 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 new plate

Protocol (384 well format, 5 µL reactions)

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 of 80% ethanol into each sample and immediately mix by pipetting up and down.
4. Place the sample plate onto the 384-well magnet and wait for 3 minutes or until the solution is clear.
5. While the plate is on the magnet, aspirate the solution (supernatant) from the sample wells and discard. Make sure 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 and wait 30 seconds.
7. While on magnet, Aspirate ethanol and discard.
8. Repeat steps 6 and 7 for a total of two ethanol washes. Especially for the last aspiration step, make sure to remove the alcohol completely.
9. Off magnet, air-dry sample at room temperature for 10 minutes. 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) and mix, and incubate at room temperature for 5 minutes.
11. Place sample plate on magnetic plate and wait 3 minutes 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 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 new plate