PCRCLEAN DX™ DNA Template Cleanup

Catalog Numbers: **C-1003-5, C-1003-50, C-1003-250, C-1003-450**

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INTRODUCTION

The PCRCLEAN DX™ purification system utilizes a proprietary magnetic bead based technology for quick and efficient, high-throughput purification of DNA from PCR and other enzyme reactions. PCRCLEAN DX™ utilizes an optimized buffer for selective binding of DNA, 120bp and larger to magnetic beads. The protocol mainly consists of binding, washing and elution steps. Primers, nucleotides, salts and enzymes in reaction mixture are removed during the binding and washing steps. The purified DNA product is essentially free of contaminants and suitable for Sanger sequencing or Next Gen sequencing library construction. Furthermore, primer dimers and adaptors can be effectively removed by using PCRCLEAN DX™.

PCRCLEAN DX™ can be used in the following applications:

PCR
Sequencing (Sanger and Next Gen library construction)
Genotyping and mutation detection
All enzymatic reaction clean up
Microarray sample preparation
Primer Walking
Cloning and all other molecular engineering

The PCRCLEAN DX™ is adaptable to various commercially available automation platforms.

PROCESS OVERVIEW

1. Add 18ul PCRCLEAN DX™ per 10ul of PCR for DNA binding to magnetic beads.
2. Separate beads and DNA from unbound contaminants.
3. Wash beads twice with 80% Ethanol to remove salt and contaminants (Fresh 70% ethanol may be used instead).
4. Elute purified DNA from magnetic beads to a new plate.

PRODUCT SPECIFICATIONS

The PCRCLEAN DX™ kit can be performed in a tube, 96 well and 384 well formats. The following table illustrates the number of clean up reactions PCRCLEAN DX™ kits can perform depending on the sample volume of PCR.

PCRCLEAN DX™	Catalog Number
PCRCLEAN DX™ - 5 mL	C-1003-5
PCRCLEAN DX™ - 50 mL	C-1003-50
PCRCLEAN DX™ - 200 mL	C-1003-200

Number of reactions:

DNA Sample Volume (uL)	**C-1003-5** (Numbers of reactions)	**C-1003-50** (Numbers of reactions)	**C-1003-200** (Numbers of reactions)
10	277	2777	11111
20	138	1388	5555
50	55	555	2222

MATERIALS

Supplied in the Kit:

PCRCLEAN DX™ magnetic bead solution

Store at 4°C upon arrival (DO NOT FREEZE), for up to 12 months
Mix the reagent well at room temperature to completely resuspend beads prior to use. It should appear visually homogenous.

To be supplied by the User:

Apparatus

Name	Recommended Model	Recommended Vendor and P/N
96-well PCR reaction plate	96-well round-bottom microtiter plate	Corning, Inc., # 3797, www.corning.com Fisher Scientific # 07-200-105, www.fishersci.com
	96 well cycling plate	ABgene Limited, # AB-0800, AB-1000, AB-1400, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S.
384-well PCR reaction plate	384 well cycling plate	Bio-Rad Laboratories, # HSP-3801, www.bio-rad.com
		ABgene product # AB-1111, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S.
Magnetic PCR plate	96 well ring stand	Ambion Inc., (acquired by Applied Biosystems), # AM10050, www.appliedbiosystems.com
	384 well [MagnaBot® 384 Magnetic Separation Device]	Promega Corporation, # V8241, www.promega.com
PCR Plate Seals	Easy Peel Heat Sealing Foil	ABgene Limited, # AB-3739 and AB-3739/s, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S.
Liquid handling robotics		
multichannel hand pipette		

Reagents

Reagents	Application
80% ethanol (or freshly made 70% ethanol)	Washing
10 mM TRIS-Acetate, pH 8.0	DNA elution
Reagent grade water	
10 mM Tris-Acetate pH 8.0, 1 mM EDTA	

PROCEDURE – 96 WELL FORMAT

1. **Determine whether or not it is necessary for a plate transfer.**

   Note: If the PCR volume multiplied by 2.8 exceeds the volume of the PCR plate, a transfer of PCR sample to a 300 uL round bottom plate is required.

2. **Gently shake the PCRCLEAN DX™ bottle to ensure a COMPLETE resuspension of any magnetic particles that may have settled. Add PCRCLEAN DX™ according to the table below:**

PCR Volume (µL)	PCRCLEAN DX™ Volume at 1.8X (µL)
10	18
20	36
50	90

   Note: Use the following equation to determine the volume of PCRCLEAN DX™:

   (Volume of **PCRCLEAN DX™** per reaction) = 1.8 x (PCR Volume)

3. **Mix PCRCLEAN DX™ and PCR sample thoroughly by pipetting up and down at least 5 times.**

   Note: After mixing, the color of the mixture should appear homogenous.

4. **Incubate the mixed samples for 5 minutes at room temperature for maximum recovery.**

   Note: This step allows the binding of PCR products 120bp and greater to the magnetic beads.

5. **Place the reaction plate onto a 96 well magnet plate for 3 minutes or wait until the**

solution is clear.

Note: Wait until the solution is clear before proceeding to the next step. Otherwise unsettled beads may become lost.

6. **Aspirate the cleared solution from the reaction plate and discard.**

Note: This step must be performed while the reaction plate is placed on the magnet plate. Avoid disturbing the settled magnetic beads. If beads are drawn into tips, eject for the beads to resettle and leave behind a few microliters of solution.

7. **Dispense 200 µL of 80% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate the ethanol and discard. Repeat for a total of two washes.**

Note: It is important to perform these steps with the reaction plate on a magnetic plate. Do not disturb the settled magnetic beads. Remove all of the ethanol from the bottom of the well to avoid ethanol carryover.

A 5 min air dry at room temperature is recommended for the evaporation of the remaining traces of ethanol. DO NOT OVERDRY THE BEADS (bead ring appears cracked) as this will significantly decrease elution efficiency.

8. **Take off the plate from the magnetic plate, add 40 µL of elution buffer (Reagent grade water, 10mM Tris-Acetate pH 8.0, or TE buffer) to each well of the reaction plate and pipette mix 5 times.**

Note: More than 40 µL of elution buffer can be used, but using less than 40 µL will require extra mixing to ensure the liquid comes into contact with the beads and may not be sufficient to elute the entire PCR product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

9. **Place the reaction plate onto a 96 well Magnetic Plate for 1 minute to separate beads from the solution.**

10. **Transfer the eluate to a new plate for storage and analysis.**

PROCEDURE - 384 WELL FORMAT

1. **Gently shake the PCRCLEAN DX™ bottle to resuspend any magnetic particles that may have settled. Add PCRCLEAN DX™ according to the following PCR volume table**:

PCR Volume (µL)	PCRCLEAN DX™ Volume (µL)
5	9
7	12.6
10	18
14	25

Note: Use the following equation to determine the volume of PCRCLEAN DX™ for a given reaction: (Volume of **PCRCLEAN DX™** per reaction) = 1.8 x (PCR Volume)

Due to the constraint of the total volume of PCR plus reagent, it is not possible to purify PCR reactions larger than 14 µL with 384 well plates (14 µL reaction + 25 µL PCRCLEAN DX™ = 39 µL).

2. **Mix PCRCLEAN DX™ and PCR sample thoroughly by pipette mixing 5 times and incubate at room temperature for 5 minutes.**

Note: The color of the mixture should appear homogenous after mixing.

3. **Place the reaction plate onto a 384 magnetic plate for 1 minute to separate the beads from solution.**

Note: Wait until the solution is clear before proceeding to the next step.

4. **Aspirate the cleared supernatant from the reaction plate and discard.**

   Note: This step should be performed while the reaction plate is placed on the 384 well magnetic plate. Do not touch the magnetic beads, which have formed a spot on the side of the well.

5. **Dispense 30 μL of 80% ethanol wash solution to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate the ethanol out and discard. Repeat for a total of two washes. Freshly made 70% ethanol may be used instead.**

   Note: It is important to perform these steps with the reaction plate on a 384 well magnetic plate. Do not disturb the separated magnetic beads. Be sure to remove all of the traces of ethanol from the bottom of the well as it is a known PCR inhibitor.

   A drying time of 5 min at room temperature is optional to ensure all traces of ethanol are removed. Take care not to over dry the bead (beads appears cracked) as this will significantly decrease DNA yield.

6. **Off the magnet plate, add 15 μL of elution buffer (Reagent grade water, Tris-Acetate pH 8.0, or TE) to each well and pipette mix 5 times.**

   A 15 μL elution volume will ensure the liquid level will be high enough to contact the magnetic beads. A greater volume of elution buffer can be used, but using less than 15 μL requires extra mixing (to ensure the liquid comes into contact with the beads) and may not fully elute the entire product. Elution is quite rapid and it is not necessary for the beads to go back into solution.

   When setting up downstream reactions, pipette the DNA from the plate while it is situated on a 384 well magnetic plate. This will prevent bead carry over (however, beads will not inhibit thermal cycling reactions).

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