omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® EquiPure gDNA Normalization Kit

M6423-00	1 x 96 preps
M6423-01	4 x 96 preps

Manual Date: July 2019
Revision Number: v5.0

For Research Use Only

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Table of Contents

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Introduction

The Mag-Bind® EquiPure gDNA Normalization Kit completely eliminates the need to aliquot DNA, saving time and tip cost. Using our proprietary Mag-Bind® Particles LRQ and binding buffer system, input DNA of various quantities is simply bound, washed and eluted to a final normalized product. The magnetic beads have a limited binding capacity and therefore allow a predefined amount of DNA to be captured and eluted.

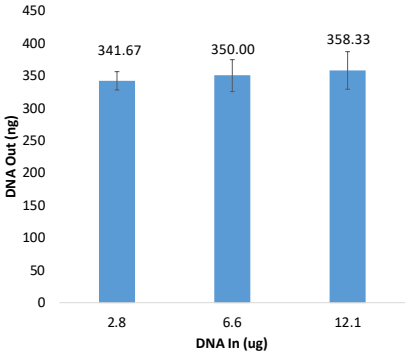
Expected Recovery: The amount of DNA that will bind to the LRQ beads depends on the size of the DNA, the amount of input and method used for purification. Adding less DNA than the recommended input amount will cause more variation in the normalized product, although DNA amounts below the recommended input amount will still bind to the Mag-Bind® Particles LRQ. **The recommended DNA input amount is 2000 ng or greater**.

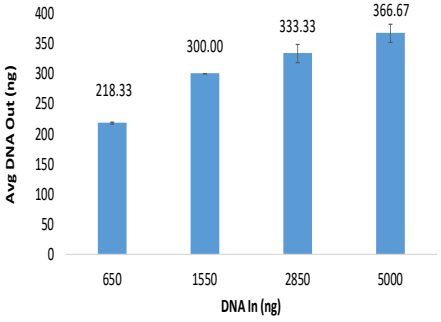
Different extraction techniques will have varying sizes of isolated genomic DNA. For best results use a magnetic bead based purification method or salt out based method for gDNA purification. Silica spin columns tend to shear gDNA into a wider fragment size which will cause more variation in the normalized product. Magnetic bead based methods results in larger fragment sizes and provide the best results.

To change your output DNA concentration, adjust the elution volume used. Do not adjust the volume of Mag-Bind® Particles LRQ added to the reaction as this will increase variation. If more DNA is required for output then double the total reaction size for the amount of binding buffer(XP2), magnetic beads, and sample size. Doubling the reaction without sufficient input amount(4000 ng>) can cause more variation for normalized DNA amount.

DNA that does not bind to the Mag-Bind® Particles LRQ can be recovered with an optional recovery protocol.

Mag-Bind® EquiPure gDNA Normalization Kit will normalize to different levels depending on several factors. **While the samples will normalize it is highly recommended to quantify 10% of samples to find normalization range.** A flourescent based dye quantification method is highly recommended.





The corresponding DNA amounts were added to 50 µL reactions and processed using the Mag Bind Equipure gDNA Normalization kit. DNA concentration measured using Promega Quantiflour dsDNA Kit. Genomic DNA was isolated using Omega Bio-tek's Mag-Bind Tissue & Blood DNA HDQ Kit(M6399).

Kit Contents

Product	M6423-00	M6423-01
Preparations	1 x 96	4 x 96
Mag-Bind® Particles LRQ	1.1 mL	4.4 mL
XP2 Binding Buffer	20 mL	80 mL
SPM Buffer	15 mL	2 x 30 mL
Elution Buffer	30 mL	125 mL
User Manual	✓	✓

Storage and Stability

All Mag-Bind® EquiPure gDNA Normalization Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles LRQ must be stored at 2-8°C. All other components should be stored at room temperature. If any precipitates form in the buffers, warm at 37°C to dissolve.

New in this Edition:

July 2019:

- XP2 Buffer has been renamed XP2 Binding Buffer. This is a name change only.

November 2018:

- SPM Wash Buffer has been renamed SPM Buffer. This is a name change only.

- Dilute SPM Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6423-00	35 mL
M6423-01	70 mL

Mag-Bind® EquiPure gDNA Normalization Kit Protocol

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device (Recommended ALPAQUA® Magnum FLX A000400)
- Vortexer
- Sealing film
- 100% ethanol
- 96-well PCR plate with a capacity of ≥260 µL and compatible with the magnetic separation device used

Before Starting:

- Prepare SPM Buffer according to Preparing Reagents section on Page 4.
- **IMPORTANT**: Protocol must be followed exactly as outlined. Do not change volumes used for input amount, amount of Mag-Bind® Particles LRQ, or other conditions. To vary DNA output concentration amount of elution buffer used can be varied.

1. Transfer up to 50 µL DNA to be normalized to a 96-well PCR plate (≥260 µL volume; not provided).

   Note: If the reaction volume is less than 50 µL, add Elution Buffer to bring the volume up to 50 µL. Do not change the reaction size.

2. Add 50 µL XP2 Binding Buffer and 10 µL Mag-Bind® Particles LRQ. Vortex or pipet up and down to mix thoroughly.

3. Let sit at room temperature for 10 minutes.

4. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles LRQ. Let sit at room temperature until the Mag-Bind® Particles LRQ are completely cleared from solution.

5. Aspirate and the supernatant. If unbound DNA is to be recovered transfer supernatant to a new 96-well PCR plate. Refer to the "Unbound DNA Recovery" protocol on Page 8. If unbound DNA is not to be recovered then discard superntant. Do not disturb the Mag-Bind® Particles LRQ during aspiration.

6. Remove the plate from the Magnetic Separation Device.

7. Add 100 µLXP2 Binding Buffer. Vortex or pipet up and down to mix thoroughly.

8. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles LRQ. Let sit at room temperature until the Mag-Bind® Particles LRQ are completely cleared from solution.

9. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles LRQ.

10. Add 150 µL SPM Buffer. ***Do not*** remove plate from the Magnetic Separation Device.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

11. Let sit at room temperature for 1 minute.

12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles LRQ.

13. Repeat Steps 10-12 for a second SPM Buffer wash step.

14. Leave the plate on the Magnetic Separation Device for 3 minutes to air dry the Mag-Bind® Particles LRQ. Remove any residual liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® Particles LRQ completely before elution. Residual ethanol may interfere with downstream applications.

15. Remove the plate from the Magnetic Separation Device.

16. Add 25-100 µL Elution Buffer. Vortex or pipet up and down to mix thoroughly.

17. Let sit at room temperature for 5 minutes.

18. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles LRQ. Let sit at room temperature until the Mag-Bind® Particles LRQ are completely cleared from solution.

19. Transfer the supernatant containing the normalized DNA to a new plate.

20. Store the DNA at -20°C.

Mag-Bind® EquiPure gDNA Normalization Kit Protocol - Unbound DNA Recovery

Materials and Equipment to be Supplied by User:

- Magnetic Separation Device (Recommended ALPAQUA® A001322)
- Vortexer
- Sealing film
- 100% ethanol
- 96-well PCR Plate with a capacity of ≥260 µL and compatible with the Magnetic Separation Device used
- Mag-Bind® Particles RQ (Cat# MBPRQ-50)
- Additional CB Buffer. CB Buffer provided with kit is enough to perform normalization protocol only.

Before Starting:

- Prepare SPM Buffer according to Preparing Reagents section on Page 4.

Complete Steps 1-4 of the Mag-Bind® EquiPure gDNA Normalization Kit Protocol on Page 5 before beginning this protocol.

1. Transfer the supernatant to a new 96-well PCR plate (not provided). Do not disturb the Mag-Bind® Particles LRQ.

2. Add 5 µL Mag-Bind® Particles RQ (not provided). Vortex or pipet up and down to mix thoroughly.

3. Let sit at room temperature for 10 minutes.

4. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

5. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles RQ.

6. Remove the plate from the Magnetic Separation Device.

7. Add 100 µL XP2 Binding Buffer. Vortex or pipet up and down to mix thoroughly.

8. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

9. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles RQ.

10. Add 150 µL SPM Buffer. ***Do not*** remove plate from the Magnetic Separation Device.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

11. Let sit at room temperature for 1 minute.

12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles RQ.

13. Repeat Steps 10-12 for a second SPM Buffer rinse step.

14. Leave the plate on the Magnetic Separation Device for 5-10 minutes to air dry the Mag-Bind® Particles RQ. Remove any residual liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® Particles RQ completely before elution. Residual ethanol may interfere with downstream applications.

15. Remove the plate from the Magnetic Separation Device.

16. Add 25-100 µL Elution Buffer. Vortex or pipet up and down to mix thoroughly.

17. Let sit at room temperature for 5 minutes.

18. Place the plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

19. Transfer the supernatant containing the DNA to a new plate.

20. Store the DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Low DNA Yields	
Low Input DNA	Use at least 500 ng amount of starting volume to achieve desired results.
No DNA eluted	
SPM Buffer was not diluted with 100% ethanol	Prepare SPM Buffer as instructed on the bottle, or refer to Page 4.
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from Mag-Bind® Particles LRQ increase A_{260}	Rely on agarose gel/ethidium bromide electrophoresis for quantification or flourescent based dye. UV based quantification methods should be avoided.
DNA sample floats out of well while loading agarose gel.	
Ethanol was not completely removed from the Mag-Bind® Particles LRQ	Completely remove any residual ethanol at Step 14. Increase the drying time in Step 14.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
SPM Buffer (40 mL)	PS014
Mag Bind Particles RQ (50 mL)	MPRQ-50

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

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Blood / Plasma

Plasmid


Cultured Cells


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NGS Clean Up


Tissue


FFPE


Fecal Matter

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innovations in nucleic acid isolation

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