omega BIO-TEK
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Product Manual

Mag-Bind® EquiPure Library Normalization Kit

M6445-00	1 x 96 preps
M6445-01	4 x 96 preps

Manual Date: April 2019
Manual Revision: v3.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

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omegabiotek
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Mag-Bind® EquiPure Library Normalization Kit

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Introduction

Many high-throughput applications, such as sequencing and genotyping, require the input DNA concentration to be within a certain range for optimal results. Traditionally, a tedious process of quantification, calculation and concentration adjustment must be carried out to normalize the DNA samples.

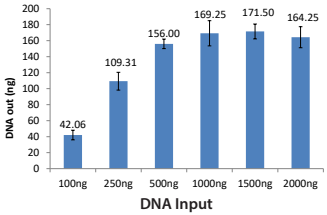
The Mag-Bind® EquiPure Library Normalization Kit completely eliminates the need to aliquot DNA, saving time and tip cost. Using our proprietary Mag-Bind® Particles LRQ and binding buffer system, input DNA of various quantities is simply bound, washed and eluted to a final normalized product. The magnetic 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. 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.

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

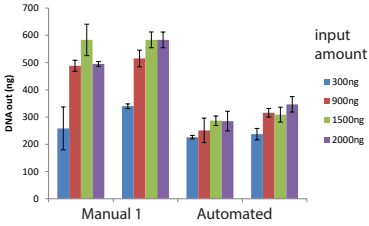
Mag-Bind® EquiPure Library Normalization Kit will normalize to different levels depending on several factors. **While the samples will normalize it is highly recommended to quantify 10% of samples to find normalization range.** A flourescent based dye quantification method is highly recommended.

DNA Input vs Output Variation



Corresponding 16S Illumina Indexed libraries DNA was added to a 50 µL reaction and purified with the Mag-Bind® EquiPure Normalization Kit. DNA amounts greater than 500 ng show a CV <10% when input range amount is greater than 500 ng.

Method vs DNA Output



Corresponding 16S Illumina Indexed libraries DNA was added to a 50 µL reaction and purified with the Mag-Bind® EquiPure Normalization Kit via manual method and Thermo Kingfisher Flex instrument. DNA amounts greater than 300 ng normalized with both methods but to different output levels.

Kit Contents

New in this Edition:
April 2019

- SPM Wash Buffer has been renamed SPM Buffer. This is a name change only. The formulation has not changed.

Product	M6445-00	M6445-01
Preparations	1 x 96	4 x 96
Mag-Bind® Particles LRQ	1.1 mL	4.4 mL
CB Buffer	35 mL	150 mL
SPM Buffer	15 mL	45 mL
Elution Buffer	30 mL	125 mL
User Manual	✓	✓

Storage and Stability

All Mag-Bind® EquiPure Library 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M6445-00	35 mL
M6445-01	105 mL

Mag-Bind® EquiPure Library 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 200 µL CB 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 µL CB 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 rinse step.

14. Leave the plate on the magnetic separation device for 5-10 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 Library 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 the 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 Library 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 CB 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

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

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Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
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