Steinbrenner Laborsysteme GmbH

magtivio

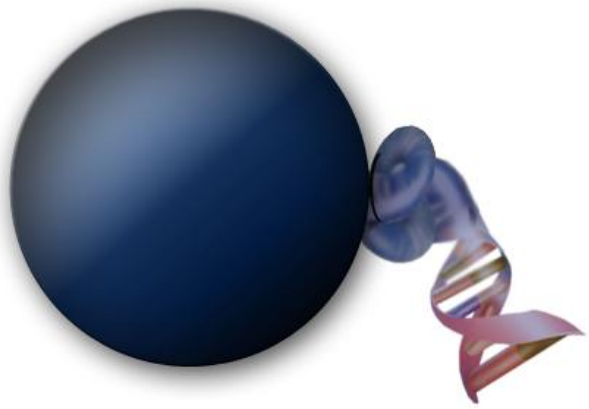
MagSi-NGS^{PREP} Plus

Art.No.

MDKT00010005

MDKT00010075

MDKT00010500



Product Manual

Version 3.1 | 13-12-2022

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Revision history		
Revision	**Release date**	**Remarks**
1.0	09/08/2018	Initial release
3.0	02-06-2022	Layout changes, revision history added
3.1	13/12/2022	New company style

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Table of Contents

1. General Information

1.1 Intended Use

MagSi-NGS^{PREP} Plus is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

MagSi-NGS^{PREP} Plus provides a convenient tool for ultra-fast and efficient purification and size selection of DNA products. DNA fragments will be bound directly onto the surface of the magnetic beads, leaving unincorporated nucleotides, primers, primer dimers, and other contaminants in solution. Finally, the DNA fragments are eluted with low salt buffer or reagent grade water.

The technology for binding of DNA fragments onto the applied magnetic nanoparticle surface does not require use of any hazardous chaotropic buffers. The purification protocols are optimized to provide high yield and purity of the recovered DNA fragments.

Depending on which protocol is used, the total preparation time is 20-30 minutes and the hands-on time necessary for the whole procedure is reduced to a minimum. The kit can be used manually and on automated workstations using single tubes, or 96- and 384-well PCR plates.

1.2 Kit specifications

MagSi-NGS^{PREP} Plus allows either non-selective binding, or size-targeted binding of double-stranded DNA fragments ranging from 80 – 1000 bp with specific reagent volume : sample volume ratio's. By increasing the volume of MagSi-NGS^{PREP} Plus, the efficiency of binding smaller fragments increases. This enables the user to selectively keep or discard undesired fragment sizes.

1.3 Basic principle

MagSi-NGS^{PREP} Plus allows for size-targeted binding of double-stranded DNA fragments ranging from 80 – 1000 bp with specific reagent volume : sample volume ratio's. The binding technology is developed to keep enzymes, salts, primers and other small nucleotides in solution.

By increasing the volume of MagSi-NGS^{PREP} Plus, the efficiency of binding smaller fragments increases. By decreasing the volume of MagSi-NGS^{PREP} Plus, the efficiency of binding smaller fragments decreases. This enables the user to selectively keep or discard undesired fragment sizes.

After binding the desired size of DNA fragments, the beads are washed twice with ethanol solutions. Traces of ethanol are removed by a brief drying step, after which the DNA fragments are released from the beads in Elution Buffer, molecular biology grade water, TRIS (10 mM, pH 8.0) or TE-buffer.

2. Materials

2.1 Kit Contents

Article Number	MDKT00010005	MDKT00010075	MDKT00010500
Kit size	5 mL	75 mL	500 mL
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2.2 Materials Supplied by the User

Consumables & Equipment	
Multichannel pipettes	20 µL and 200 µL
PCR plates	96-well PCR Plates, (suggested: ABgene, Cat.No.: AB-0800, AB-1000 or AB-1400) 384-well PCR Plates, (suggested: ABgene, Cat.No.: AB-1111)
Magnetic separator	MM-Separator M96 (Art.No.: MD90002): Magnetic separator for 96-well microplates and PCR plates. MM-Separator 96 PCR (Art.No.: MDMG0005): Magnetic separator for 96-well PCR plates, U and V-bottom microplates. suitable for automated processes MM-Separator 384 PCR (Art.No.: MDMG0006): Magnetic separator for 384-well PCR. suitable for automated processes
Reagents	
Pure Ethanol p.a.	VWR cat# 1.00013.1000
Elution buffer	Molecular biology grade water, TRIS (10 mM, pH 8.0) or TE-buffer

3. Kit usage

3.1 Storage Conditions

MagSi-NGS^{PREP} Plus should be stored at 2-8°C. When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Preparation of reagents

- Prepare an ethanol solution freshly (!):
  - For Protocol 4.2, prepare a 70% ethanol solution.
  - For protocol 4.3, 4.4 and 4.4, prepare an 85% ethanol solution.
- Before use, vortex MagSi-NGS^{PREP} Plus intensively into a homogeneous suspension.

3.3 Safety instructions

Take appropriate safety measures, such as wearing a suitable lab coat, disposable gloves, and protective goggles. Follow local legal requirements for working with biological materials.

More information is found in the safety data sheets (SDS), available at www.magtivio.com under each magtivio kit and kit component.

Infectious potential of liquid waste left over after using MagSi-NGS^{PREP} Plus was not tested. Even though contamination of waste with residual infectious material is unlikely, it cannot be excluded completely. Therefore, liquid waste should be handled as being potentially infectious, and discarded according to local safety regulations.

4. Protocols

4.1 Summary of MagSi-NGS^{PREP} Plus uses and protocols

A. General-purpose DNA clean-up (Protocol 4.2)

For clean-up of DNA fragment (without size selection) following all DNA manipulation procedures*. MagSi-NGS^{PREP} Plus efficiently captures and purifies all double stranded DNA fragments greater than about 100 bp in size.

This protocol is applicable to:

- PCR amplification procedures
- cDNA constructions
- DNA restriction digestions
- General purpose DNA manipulation, concentration, and buffer exchange procedures

B. DNA Size selection

Left Side Size Selection (Protocol 4.3)

This protocol removes DNA fragments below a target size. Target DNA is bound to the beads, washed and eluted. This protocol is applicable in various steps of NGS library preparation procedures.

Right Side Size Selection (Protocol 4.4)

This protocol removes DNA fragments above a target size. DNA above the target size is bound to the beads, leaving target DNA in solution. In a second binding step, target DNA is bound to the beads, washed and eluted.

Double Sided Size Selection (Protocol 4.5)

This protocol removes DNA fragments above and below target size range. DNA above the target size is bound to the beads, leaving target DNA in solution. In a second binding step, DNA below target size is left in solution while target DNA is bound to the beads, washed and eluted.

* MagSi-NGS^{PREP} Plus can replace the use of Agencourt® AMPure® XP in SPRI procedures without affecting protocols and automation.

4.2 Clean-up of enzymatic reactions

1. Before use, vortex MagSi-NGS^{PREP} Plus to fully resuspend the beads.
2. Add MagSi-NGS^{PREP} Plus according to the table below; mix by pipetting up and down until a homogeneous suspension is obtained. Incubate for 5 minutes to allow beads to bind the DNA.

Reaction volume (µL)	MagSi-NGS^{PREP} Plus volume (µL)
10	18
15	27
20	36
25	45
50	90
For different reaction volumes, use the following equation: Volume of MagSi-NGS^{PREP} Plus = 1.8 x Reaction Volume	

3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until a clear solution is obtained.
4. Remove the cleared supernatant from the beads and discard. This step must be performed while the plate is on the magnet. Remove the supernatant as much as possible, but make sure you do not disturb the magnetic beads.
5. Add 180 µL EtOH 70% and incubate for 30 seconds to allow the beads to settle to the magnet again. This step can be performed while the plate is placed on the magnet. (It is not necessary to resuspend the beads).
6. Discard the supernatant. This step must be performed while the plate is on the magnet.
7. Repeat steps 5-6 once more for a total of 2 washing steps.
8. Air-dry the magnetic particles for approximately 5 minutes. This step can be performed while the plate is placed on the magnet.
9. Remove the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10x and incubate for 2 minutes to elute.
10. Place the sample plate on the magnetic separator for 1 minute to collect the magnetic beads.
11. Transfer the supernatant to the final plate. This step must be performed while the plate is on the magnet. Leave 5 µL liquid behind to prevent transfer of beads into the final plate.

4.3 Left Side Size Selection

Decreasing the ratio of MagSi-NGS^{PREP} Plus volume to sample volume for Left Side Selection will decrease the efficiency of binding smaller fragments (see Figure 1).

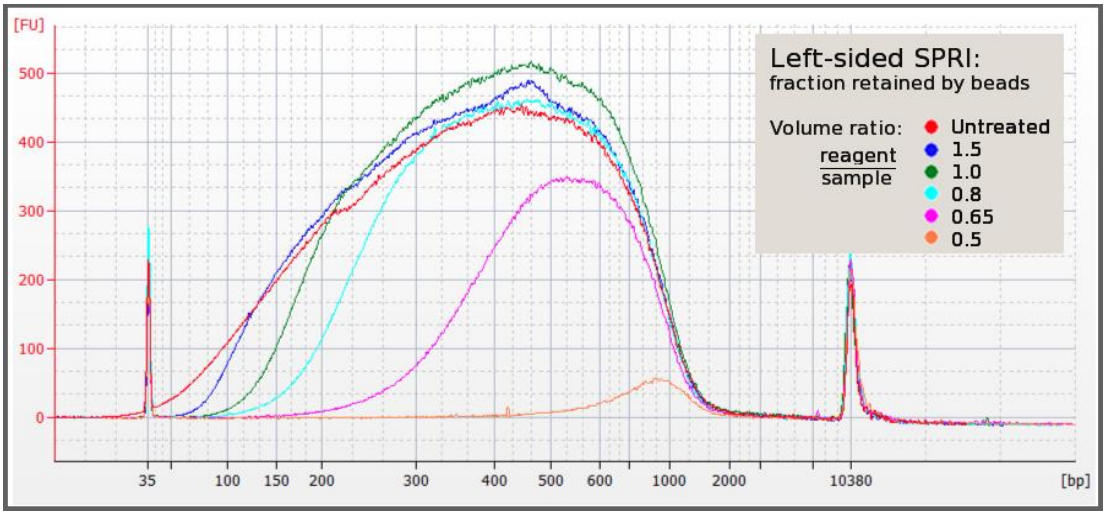

Figure 1: Left Side Size Selection with given ratios of MagSi-NGS^{PREP} Plus volume to sample volume

Volume of sample * Ratio = Volume of MagSi-NGS^{PREP} Plus

Example: 50 µL * 0.65 = 32.5 µL of MagSi-NGS^{PREP} Plus

1. Before use, vortex MagSi-NGS^{PREP} Plus to fully resuspend the beads.
2. Add the required volume of MagSi-NGS^{PREP} Plus for the desired ratio to the sample. Mix by pipetting 10x until a homogeneous suspension is obtained and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until a clear solution is obtained.
4. Remove the cleared supernatant from the beads and discard. This step must be performed while the plate is on the magnet. Remove the supernatant as much as possible, but make sure you do not disturb the magnetic beads.
5. Add 180 µL EtOH 85% and incubate for 30 seconds to allow the beads to settle to the magnet again. This step can be performed while the plate is placed on the magnet.
6. Discard the supernatant. This step must be performed while the plate is on the magnet.
7. Repeat steps 5-6 once more for a total of 2 washing steps.
8. Air-dry the magnetic particles for approximately 5 minutes. This step can be performed while the plate is placed on the magnet.
9. Remove the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10x and incubate for 2 minutes to elute.
10. Place the sample plate on the magnetic separator for 1 minute to collect the magnetic beads.

11. Transfer the eluate (size selected sample) to the final plate. This step must be performed while the plate is on the magnet. Leave 2-5 µL liquid behind to prevent transfer of beads into the final plate.

4.4 Right Side Size Selection

Increasing the ratio of MagSi-NGS^{PREP} Plus volume to sample volume for Right Side Selection will increase the efficiency of removing larger fragments (see Figure 2).

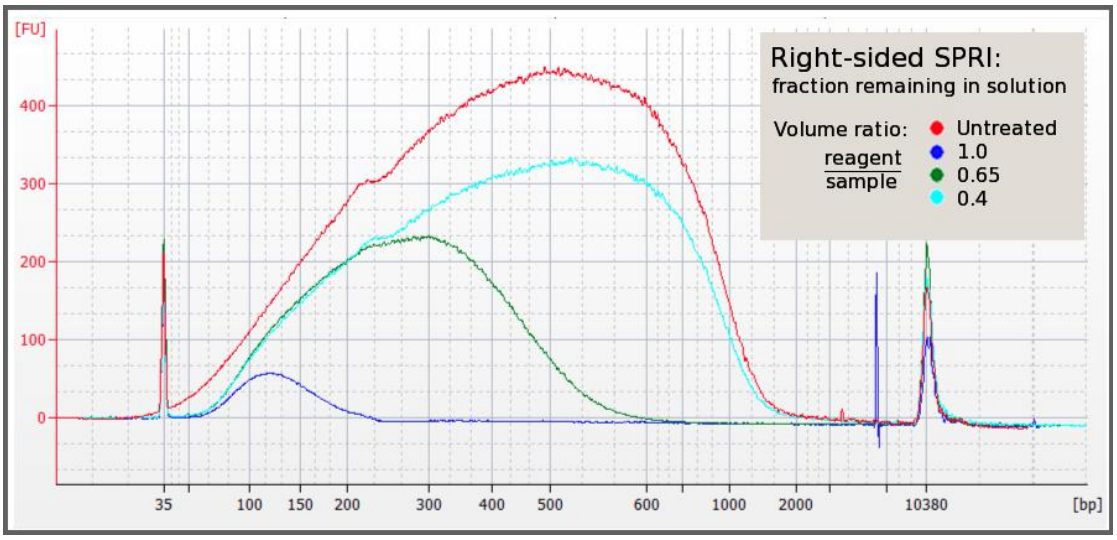

Figure 2: Right Side Size Selection with given ratios of MagSi-NGS^{PREP} Plus volume to sample volume

Volume of sample * Ratio = Volume of MagSi-NGS^{PREP} Plus

Example: 50 µL * 0.65 = 32.5 µL of MagSi-NGS^{PREP} Plus

1. Before use, vortex MagSi-NGS^{PREP} Plus to fully resuspend the beads.
2. Add the required volume of MagSi-NGS^{PREP} Plus for the desired ratio to the sample. Mix by pipetting 10x until a homogeneous suspension is obtained and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until a clear solution is obtained.
4. Transfer the cleared supernatant from the beads to a new container. Be careful not to aspirate beads, as these contain the undesired larger fragment sizes.
5. Add the required volume of MagSi-NGS^{PREP} Plus using the following formula:

Initial sample volume * (1.8 – Right Side ratio) = Volume of MagSi-NGS^{PREP} Plus

Mix by pipetting 10x until a homogeneous suspension is obtained and incubate for 5 minutes.

6. Place the sample plate on the magnetic separator to collect the magnetic beads until a clear solution is obtained. Collection times may vary; a larger initial sample volume, higher MagSi-NGS^{PREP} ratio or weaker magnet will result in longer collection times.

7. Remove the cleared supernatant from the beads and discard. This step must be performed while the plate is on the magnet. Remove the supernatant as much as possible, but make sure you do not disturb the magnetic beads.

8. Add 180 µL EtOH 85% and incubate for 30 seconds to allow the beads to settle to the magnet again. This step can be performed while the plate is placed on the magnet.

9. Discard the supernatant. This step must be performed while the plate is on the magnet.

10. Repeat steps 8-9 once more for a total of 2 washing steps.

11. Air-dry the magnetic particles for approximately 5 minutes. This step can be performed while the plate is placed on the magnet.

12. Remove the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10x and incubate for 2 minutes to elute.

13. Place the sample plate on the magnetic separator for 1 minute to collect the magnetic beads.

14. Transfer the eluate (size selected sample) to the final plate. This step must be performed while the plate is on the magnet. Leave 2-5 µL liquid behind to prevent transfer of beads into the final plate.

4.5 Double Sided Size Selection

The Left Side Selection ratio is always greater than the Right Side Selection ratio. In the first step (Right Side Selection), increasing the ratio of MagSi-NGS^{PREP} Plus volume to sample volume will increase the efficiency of removing larger fragments. In the second step (Left Side Selection), decreasing the ratio of MagSi-NGS^{PREP} Plus volume to sample volume will increase the efficiency of removing smaller fragments (see Figure 3).

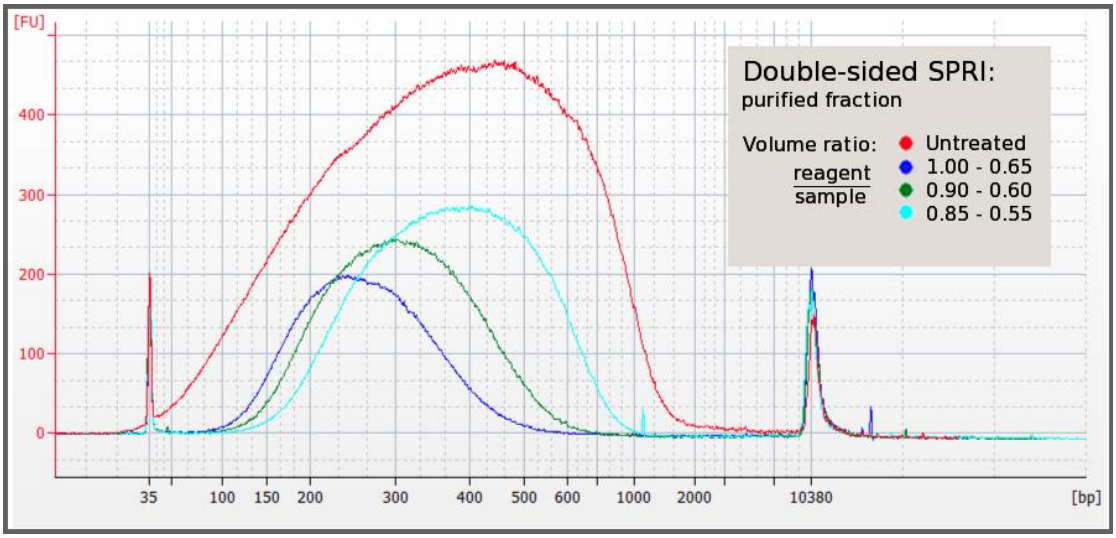

Figure 3: Double Sided Size Selection with given ratio's of MagSi-NGS^{PREP} Plus volume to sample volume

Volume of sample * Ratio = Volume of MagSi-NGS^{PREP} Plus

Example: 50 µL * 0.65 = 32.5 µL of MagSi-NGS^{PREP} Plus

1. Before use, vortex MagSi-NGS^{PREP} Plus to fully resuspend the beads.
2. Add the required volume of MagSi-NGS^{PREP} Plus for the desired Right Side Ratio to the sample. Mix by pipetting 10x until a homogeneous suspension is obtained and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until a clear solution is obtained.
4. Transfer the cleared supernatant from the beads to a new container. Be careful not to aspirate beads, as these contain the undesired larger fragment sizes.
5. Add the required volume of MagSi-NGS^{PREP} Plus using the following formula:

Initial sample volume * (Left Side ratio – Right Side ratio) = Volume of MagSi-NGS^{PREP} Plus

Mix by pipetting 10x until a homogeneous suspension is obtained and incubate for 5 minutes.

6. Place the sample plate on the magnetic separator to collect the magnetic beads until a clear solution is obtained. Collection times may vary; a higher initial sample volume, higher MagSi-NGS^{PREP} Plus ratio or weaker magnet will result in longer collection times.
7. Remove the cleared supernatant from the beads and discard. This step must be performed while the plate is on the magnet. Remove the supernatant as much as possible, but make sure you do not disturb the magnetic beads.
8. Add 180 µL EtOH 85% and incubate for 30 seconds to allow the beads to settle to the magnet again. This step can be performed while the plate is placed on the magnet.
9. Discard the supernatant. This step must be performed while the plate is on the magnet.
10. Repeat steps 8-9 once more for a total of 2 washing steps.
11. Air-dry the magnetic particles for approximately 5 minutes. This step can be performed while the plate is placed on the magnet.
12. Remove the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10x and incubate for 2 minutes to elute.
13. Place the sample plate on the magnetic separator for 1 minute to collect the magnetic beads.
14. Transfer the eluate (size selected sample) to the final plate. This step must be performed while the plate is on the magnet. Leave 2-5 µL liquid behind to prevent transfer of beads into the final plate.

5. Troubleshooting

Problem	Possible cause	Suggestion
Low recovery of DNA	Insufficient binding of DNA	- Increase pipette mixing steps - Increase binding incubation time
	Overdrying of beads	- Decrease drying time to a minimum for removal of traces of ethanol. Visually inspect for leftover liquid.
	Insufficient EtOH removal after washing steps	- Make sure to discard all wash solution - Increase drying time
	Insufficient elution	- Increase pipette mixing steps - Increase elution incubation time
Insufficient removal of unwanted reaction products	Unwanted products in wells or on beads	- For washing, use the maximum working volume - Increase pipette mixing steps for washing
Magnetic beads in final eluate	Carryover of beads into final container	- Leave 5 µL of liquid behind to prevent beads from being aspirated - Decrease aspiration speed of pipetting
Undesired fragment sizes remaining after Size Selection	Insufficient mixing of sample and MagSi-NGSPREP Plus	- After dispensing onto sample, immediately mix to prevent differences in buffer concentration within the sample - Increase mix steps after addition of MagSi-NGSPREP Plus
	Insufficient binding efficiency in removal of undesired fragments	- Increase incubation time for binding of DNA fragments
	Carryover of beads with undesired fragments	- Increase incubation time for magnetic separation - Repeat magnetic separation and transferof samples
	Reagents in the sample that effect size cut-off (e.g. polyethylene glycol, MgCl2)	- Adjust Ratio for size selection - Remove reagents from sample with Protocol 4.1, then proceed with size selection

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