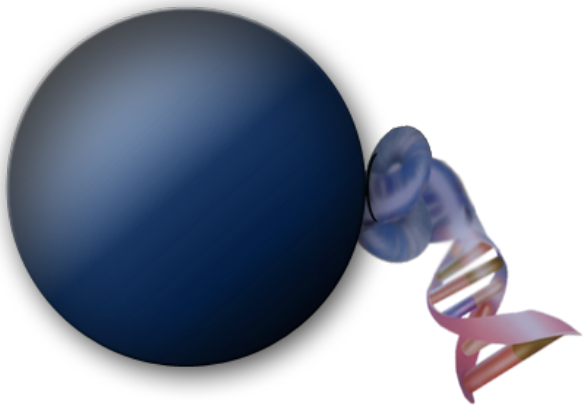
MagnaMedics
Move to Simplicity

MagSi-NGS^{PREP} Plus

Art.No.

MDKT0001



Product Manual

Version 1.0 | 03/09/2015

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Move to Simplicity

MagSi-NGS^{PREP} Plus

Clean-up and size selection in sample preparation for NGS library construction

This product is for R&D use only. Not for drug, household or other uses. For more information, please consult the appropriate Material Safety Data Sheet (MSDS), available on our website at www.magnamedics.com

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1. Introduction

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

A. General-purpose DNA clean-up (Protocol 4.1)

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 4.2)

  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 4.3)

  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 4.4)

  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.

[1] Agencourt® and AMPure® XP are registered trademarks by Beckman Coulter, Inc.

[2] All other trademarks cited here are the property of their respective owners.

1.2 General information

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.

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.

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.

The kit is stable for 1 year when stored at 2-8°C.

2. MagSi-NGS^{PREP} Plus contents

Article Number	MDKT00010005	MDKT00010075	MDKT00010500
Kit size	5 mL	75 mL	500 mL
Product Manual	1	1	1

2.1 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 (MagnaMedics, Art.No.: MD90002): Magnetic separator for 96-well microplates and PCR plates. MM-Separator PCR strip adapter (MagnaMedics, Art.No.: MD90003): Adapter for manual use with 8- and 12-tube PCR strips and is complementary to MM-Separator M96. MM-Separator 96 SBS (MagnaMedics, Art.No.: MD90005): Magnetic separator for 96-well microplates and PCR plates, suitable for automated processes MM-Separator 384 SBS (MagnaMedics, Art.No.: MD90006): Magnetic separator for 384-well microplates and PCR plates. 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 Preparations before use

- Prepare an ethanol solution freshly (!):

  For Protocol 4.1, prepare a **70% ethanol solution**.

  For protocol 4.2, 4.3 and 4.4, prepare a **85% ethanol solution**.

- Before use, vortex the MagSi-NGS^{PREP} Plus intensively into a homogeneous suspension.

3.2 Handling guidelines for MagSi-NGS^{PREP} Plus

- Drying time may vary due to differences in the laboratory environment. Optionally increase the drying time a little until the wells appear to be dry. Caution: Do not over-dry the beads, this can result in loss of recovery.
- When transferring purified samples, preferably leave 2-5 µL liquid behind in order to prevent carryover of magnetic beads into the final plate. If beads are present in final sample, perform the separation again.

3.3 Safety information

Wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online at www.magnamedics.com.

4. Protocols

4.1 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.

4.2 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).

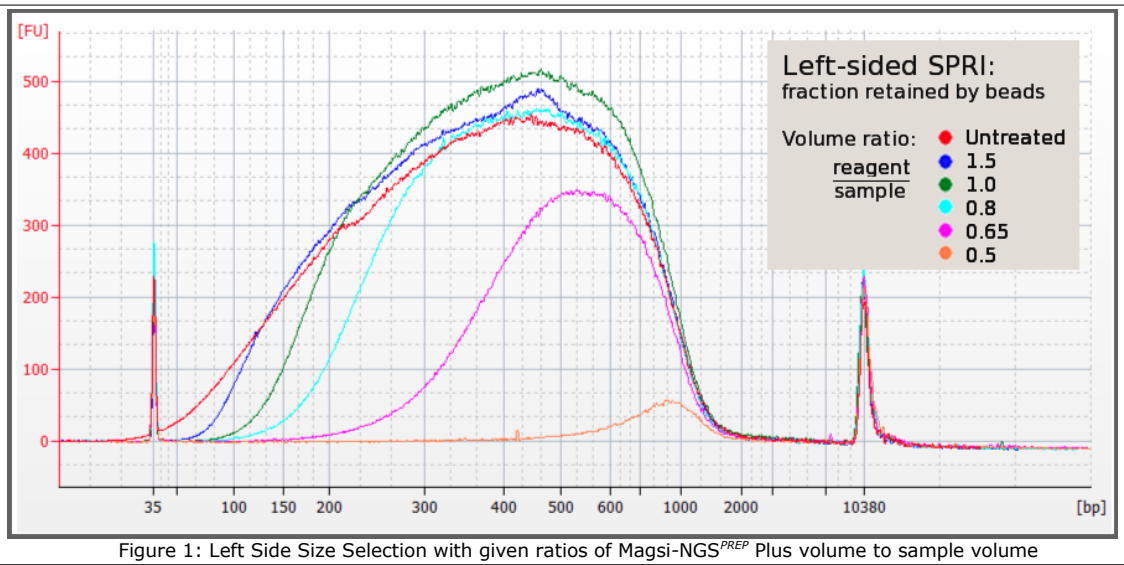

Figure 1: Left Side Size Selection with given ratios of Magsi-NGS^{PREP} Plus volume to sample volume

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.

4.3 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).

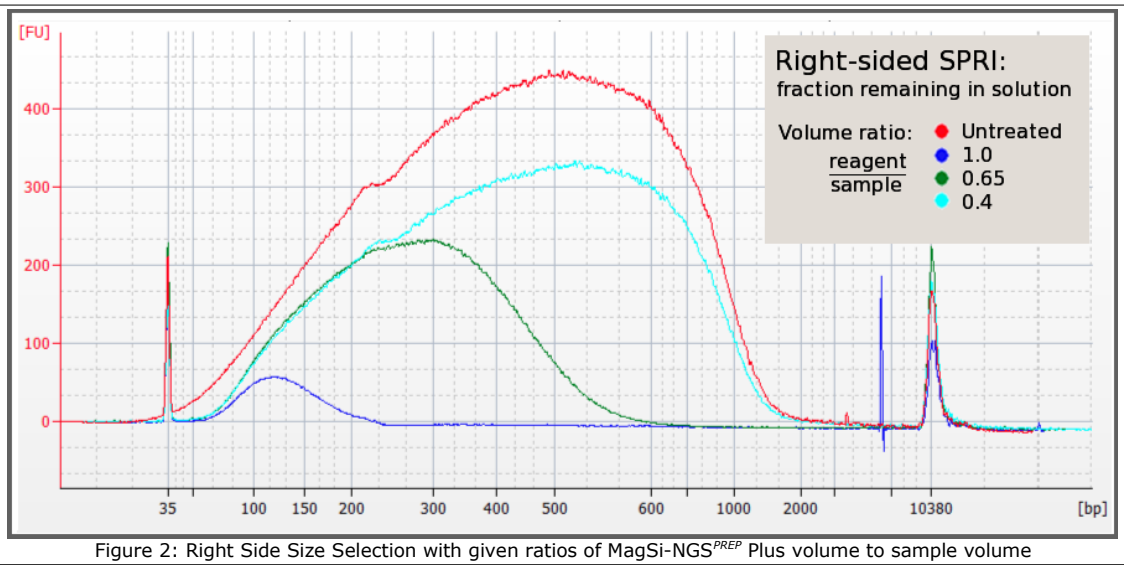

Figure 2: Right Side Size Selection with given ratios of MagSi-NGS^{PREP} Plus volume to sample volume

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 * (1.8x – 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, bigger 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.

4.4 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).

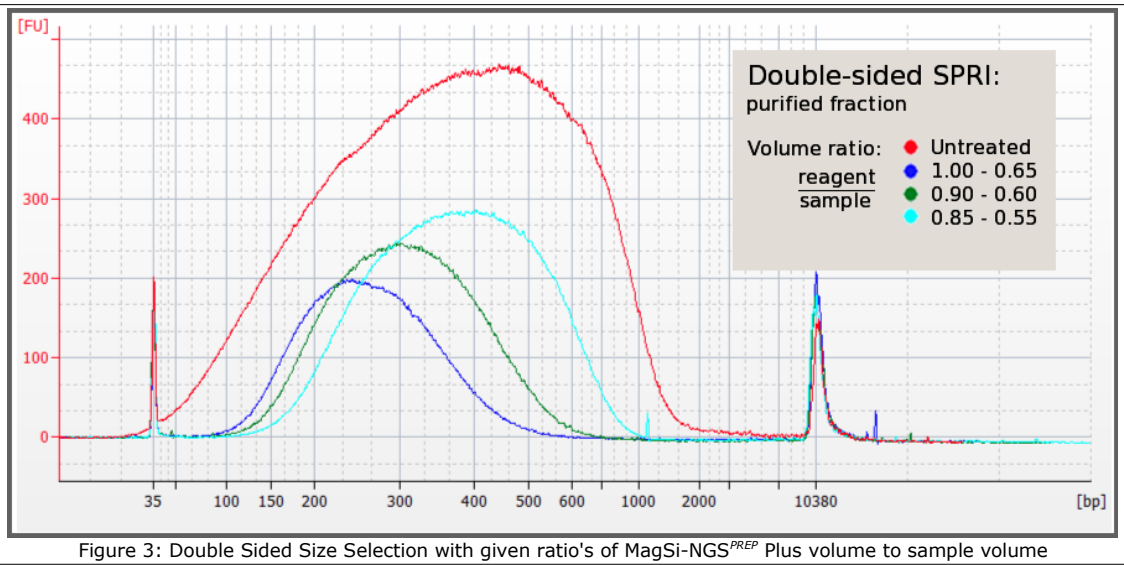

Figure 3: Double Sided Size Selection with given ratio's of MagSi-NGS^{PREP} Plus volume to sample volume

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

Troubleshooting guidelines for MagSi-NGS^{PREP} Plus

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-NGS^{PREP} Plus	- After dispensing onto sample, immediately mix to prevent differences in buffer concentration within the sample - Increase mix steps after addition of MagSi-NGS^{PREP} 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, $MgCl_2$)	- Adjust Ratio for size selection - Remove reagents from sample with Protocol 4.1, then proceed with size selection

MagnaMedics Diagnostics B.V.

IVD, Food-Diagnostics, Life-Science Products,

Laboratory & Production

De Asselen Kuil 20a

6161 RD Geleen (The Netherlands)

Tel: +31-(0)46-820 0206

Fax: +31-(0)46-410 6825

E-mail: info@magnamedics.com

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