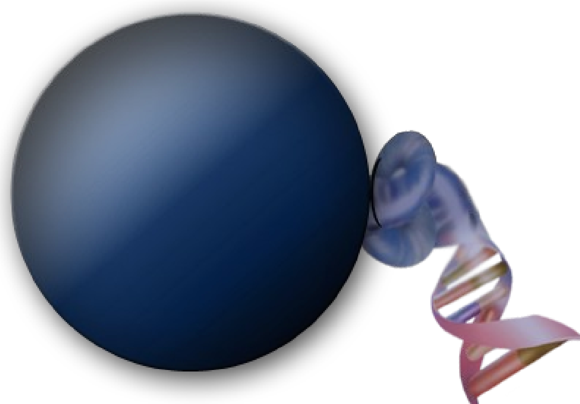
MagSi-NGS^{PREP}

Art.No.
MD60021 – MD61021



Product Manual

Version 1.2 | 09/04/2014

MagnaMedics
Move to Simplicity

MagSi-NGS*PREP*

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.

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1. General Information

MagSi-NGS^{PREP} 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} allows selective binding of DNA with a size cut-off between 100bp and 1kb with specific reagent volume:sample volume ratio's. By increasing the volume of MagSi-NGS^{PREP}, 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.

When working with chemicals, always 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 on-line in convenient and compact PDF format at www.magnamedics.com.

2. MagSi-NGS^{PREP} contents

Article Number	MD60021	MD61021
Kit size	5 mL	75 mL
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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	Magnetic Separator M96 (MoBiTec, Art.No.: PR-MAGMS02): Magnetic separator for 96-well microplates and PCR plates. Magnetic Separator for PCR Strips (MoBiTec, Art.No.: PR-MAGMS03): Adapter for manual use with 8- and 12-tube PCR strips and is complementary to MM-Separator M96. MM-Separator 96 SBS (MoBiTec, Art.No.: PR-MAGMS05): Magnetic separator for 96-well microplates and PCR plates, suitable for automated processes MM-Separator 384 SBS (MoBiTec, Art.No.: PR-MAGMS06): 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	Reagent grade water, TRIS pH 8.0, 0.1mM EDTA, or standard 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* intensively into a homogeneous suspension.

3.2 Handling guidelines for MagSi-NGS*PREP*

- 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. Careful: Do not over-dry the beads, this can result in loss of recovery/signal intensity.
- When transferring purified samples, preferably leave a minimum of 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.

4. Protocols

MagSi-NGS^{PREP} can be used invarious sample preparation processes. The overview below explains how the kit protocols can be used for different applications.

A. Clean-up steps in DNA library preparation for NGS applications (Protocol 4.1):

- PCR clean-up

  For purification of PCR products (>80 bp). This protocol uses a volume of MagSi-NGS^{PREP} equal to the Sample Volume * 1.8

- Clean-up steps in genomic library preparation kits

  For other clean-up steps in DNA library preparation protocols, use the volumes of magnetic beads as mentioned in your library prep kit manual.

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.

- Right Side Size Selection (Protocol 4.3)

  This protocol removes DNA fragments above a target size. DNA above 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 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.

4.1 Clean-up of enzymatic reactions

1. Before use, vortex **MagSi-NGS^{PREP}** to fully resuspend the beads.

2. Add **MagSi-NGS^{PREP}** 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} 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} = 1.8 x Reaction Volume	

3. Place the sample plate on the magnetic separator for 3 minutes to collect the magnetic beads until a clear solution is obtained.

4. Discard the cleared supernatant from the beads.
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 **200 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. Add **40 µL Elution Buffer**. Incubate for 2 minutes to elute.
Elution is fast but can be facilitated by pipetting up and down 7 times.

10. 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

Increasing the ratio of MagSi-NGS^{PREP} volume to sample volume will increase the efficiency of binding smaller fragments (see Table 2).

Table 2: Left Side Size Selection with given ratio of Magsi-NGS^{PREP} volume to sample volume

Ratio of MagSi-NGS^{PREP} (µL) to sample (µL)	Desired size of DNA fragments
0.4x	>1000
0.45x	>800
0.55x	>600
0.6x	>500
0.7x	>400
0.8x	>300
0.9x	>200
1.5x	>100
volume of sample * ratio = volume of MagSi-NGS^{PREP} For example: 50 µL * 0.65x ratio = 32.5 µL of MagSi-NGS^{PREP}	

1. Before use, vortex **MagSi-NGS^{PREP}** to fully resuspend the beads.
2. Add the required volume of **MagSi-NGS^{PREP}** for the desired ratio to the sample. Mix by pipetting 10x and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 3 minutes to collect the magnetic beads until a clear solution is obtained.
4. Discard the cleared supernatant from the beads.
   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. Add **40 µL Elution Buffer**. Incubate for 2 minutes to elute.
   Elution is fast but can be facilitated by pipetting up and down 7 times.
8. 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 Right Side Size Selection

Increasing the ratio of MagSi-NGS^{PREP} volume to sample volume for Right Side Selection will decrease the efficiency of binding larger fragments (see Table 3).

Table 3: Right Side Size Selection with given ratio of Magsi-NGS^{PREP} volume to sample volume

Ratio of MagSi-NGS^{PREP} (µL) to sample (µL)	Desired size of DNA fragments
0.4x	<1000
0.45x	<800
0.55x	<600
0.6x	<500
0.7x	<400
0.8x	<300
0.9x	<200
1.5x	<100
volume of sample * ratio = volume of MagSi-NGS^{PREP} For example: 50 µL * 0.65x ratio = 32.5 µL of MagSi-NGS^{PREP}	

1. Before use, vortex **MagSi-NGS^{PREP}** to fully resuspend the beads.
2. Add the required volume of **MagSi-NGS^{PREP}** 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 3 minutes to collect the magnetic beads until a clear solution is obtained.
4. Transfer the cleared supernatant from the beads to anew container.
   Be careful not to aspirate beads, as these contain the undesired larger fragment sizes.
5. Add the required volume of **MagSi-NGS^{PREP}** using the following formula:

   Initial sample volume * (1.8x – initial ratio) = volume of MagSi-NGS^{PREP}

   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. Discard the supernatant.
   This step must be performed while the plate is on the magnet.
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. Add **40 µL Elution Buffer**. Incubate for 2 minutes to elute.
    Elution is fast but can be facilitated by pipetting up and down 7 times.
11. Transfer the supernatant.
    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.

4.4 Double Sided Size Selection

The Left Side Selection ratio is always greater than the Right Side Selection ratio. Increasing the ratio of MagSi-NGS^{PREP} volume to sample volume for Right Side Selection will decrease the efficiency of binding larger fragments, while decreasing the ratio for Left Side Selection will decrease the efficiency of binding smaller fragments (see Table 4).

Table 4: Double Sided Size Selection with given ratio's of Magsi-NGS^{PREP} volume to sample volume

Ratio´s (Left - Right)	Desired size of DNA fragments
1.5x – 0.4x	100 - 1000
0.9x – 0.45x	200 - 800
0.8x – 0.55x	300 - 600
0.7x – 0.6x	400 - 500
volume of sample * ratio = volume of MagSi-NGS^{PREP} For example: 50 µL * 0.65x ratio = 32.5 µL of MagSi-NGS^{PREP}	

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

   Initial sample volume * (Left Ratio – Right Ratio) = volume of MagSi-NGS^{PREP}

   Mix by pipetting 10x 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}** ratio or weaker magnet will result in longer collection times.
7. Discard the supernatant.
   This step must be performed while the plate is on the magnet.
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. Add **40 µL Elution Buffer**. Incubate for 2 minutes to elute.
    Elution is fast but can be facilitated by pipetting up and down 7 times.
11. Transfer the supernatant.
    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.

5. Troubleshooting

Table 5: Troubleshooting guidelines for MagSi-NGS^{PREP}

Problem	Possible cause	Suggestion
Low recovery of DNA	Insufficient binding of DNA	- Increase pipette mixing steps - Increase binding incubation time
	Insufficient EtOH removal after washing steps	- Make sure to discard all wash solution - Increase drying time (maximum 10 minutes)
	Overdrying of beads	- Decrease drying 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}	- Increase mixing steps after addition of MagSi-NGS^{PREP}
	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

distributed by

MoBiTec GmbH

Molecular Biotechnology

Lotzestrasse 22a

37083 Goettingen, Germany

Tel: +49 551 707 22 0

Fax: +49 551 707 22 22

E-mail: info@mobitec.com

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