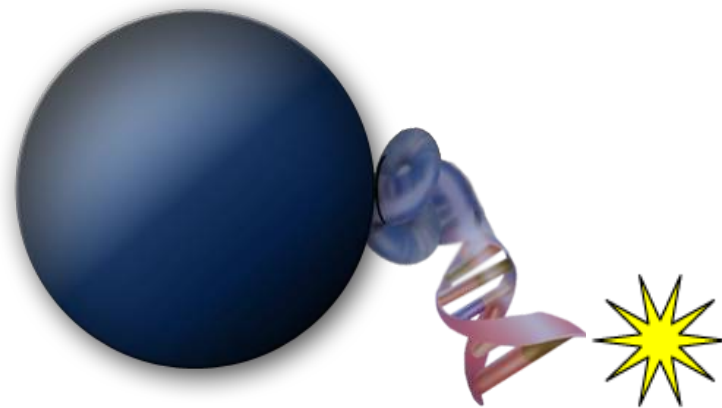




MagSi-DNA clean^{FIX}

Manual





MagnaMedics



MagSi-DNA clean^{FIX}

Dye-Terminator Removal and PCR clean-up

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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General information

MagSi-DNA clean^{FIX} provides a convenient tool for ultra-fast and efficient purification of DNA products from 80 bp up to 30 kb. The kit can be used manually and on automated workstations using single tubes, or 96- and 384-well PCR plates. 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 isolation protocol as well as all buffers are optimized to provide high yield and purity of the recovered DNA fragment. The "hands-on time" necessary for the whole procedure is reduced to a minimum.

The kit is designed to address two applications:

1. **Dye-Terminator Removal from DNA cycle sequencing reactions** prior to read out in DNA sequencers (protocol 1A and 1B)
2. **Purification and concentration of DNA fragments**, especially crude PCR mixes to be cleaned up for DNA cycle sequencing reaction (protocol 2A and 2B)

The protocols act as independent modules which can be combined individually by the user on any working platform. For example, after purification of 20 μ L PCR products in 96-well PCR plates (protocol 2A), sequencing reactions of 10 μ L can be prepared and purified in 384-well PCR plates (protocol 1B) for DNA analysis.

Purified PCR products (protocol 2A and 2B) are ready-to-use in various downstream application such as:

- Digestion with restriction enzymes
- Hybridization
- Labelling
- Cloning
- DNA Sequencing
- In vitro Transcription



MagSi-DNA clean^{FIX} contents

Kit size	400/800 purifications	5K/10K purifications
Article Number	MD60013	MD60014
MagSi-DNA clean ^{FIX} particle mix	4 mL	50 mL
MagSi-DNA clean Buffer P	12 mL	150 mL
Manual	1	1

*the number of preparations is based on 384 well format protocol using 5 µL of MagSi-DNA clean^{FIX} particle mix

Materials Supplied by the User

Consumables & Equipment	
Multichannel pipettes	20 µL and 200 µL
PCR plates	Full Skirt 96-well PCR Plates, (Axygen Inc., Cat.No.: PCR-96-FS-C) 384-well PCR Full Skirt Plates, (Axygen Inc., Cat.No.: PCR-384-C)
Magnetic separator	MM-Separator M96 (MagnaMedics, Art.No.: MD90002): Magnetic separator for 96-well microplates and PCR plates 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	
Isopropanol p.a.	VWR cat# 1.00983.1011
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
Mineral oil (optional)	Carl Roth, cat# HP50.1

- *Alcohol mix**: 42.5% isopropanol (p.a.), 42.5% Ethanol (p.a), 15% ddH₂O to be prepared by the user.



Kit usage

The manual contains different protocols as described in the table below.

Dye-Terminator Removal			
Protocols	Plate format	sample volume	working volume
1A: sequence clean-up	96-well PCR plate	10-20 μ L	49-73 μ L
1B: sequence clean-up	384-well PCR plate	5-10 μ L	23-35 μ L
PCR clean-up			
Protocols	Plate format	sample volume	working volume
2A: PCR clean-up	96-well PCR plate	10-20 μ L	40-60 μ L
2B: PCR clean-up	384-well PCR plate	5-10 μ L	20-30 μ L

Preparation time is approximately 25 minutes.

The kit components are stable for at least 1 year after production date when stored in the appropriate conditions. 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 online in convenient and compact PDF format at www.magnamedics.com.



Dye-Terminator Removal from DNA cycle sequencing reactions of PCR-products and Plasmids after use ABI Prism™ terminator Kits

Protocol 1A (96-well PCR plate, for 10-20 µL sequencing reaction mix):

- 1. Before use, shake MagSi-DNA clean^{FIX} particle mix to fully resuspend the beads.**
- 2. Add 10 µL MagSi-DNA clean^{FIX} magnetic particle mix to each sample.**
- 3. Add alcohol mix* (see Kit Contents, page 3) according to the table below; mix well by pipetting up and down 10 times.**

Sequencing reaction volume (µL)	Volume of Alcohol mix (µL)
10	29
20	43

- 4. Incubate for 3 minutes at room temperature.**
- 5. Place the sample plate on the magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
- 6. Washing of the magnetic beads: Add 100 µL alcohol mix*** and resuspend the magnetic beads by pipetting up and down 15 times.
- 7. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Repeat steps 6-7 once more for a total of 2 washing steps.
- 8. Remove the samples from the magnet and air-dry the magnetic particles for 5 minutes.**
- 9. Elution of the DNA fragments: Add 40 µL Elution Buffer** to the magnetic bead sample and mix by pipetting up and down 10 times. Incubate for 2 minutes at room temperature.
- 10. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container, but leave ±5 µL liquid behind to prevent transfer of beads.**

Technical notes:

An elution volume of 40 µL is sufficient to ensure proper elution of the DNA fragments. A higher volume (up to 100 µL) can be used, but a minimum volume of 30 µL is needed to ensure contact between the liquid and the magnetic particles. For Elution buffer, reagent grade water or 0.1 mM EDTA can be used, depending on the preferred signal intensity.

For better stability of the purified sample, add mineral oil (Carl Roth, HP50.1) to the purified sample, optionally followed by short centrifugation to assure proper sealing.

Another possible optimization is to perform the elution in 2.25 mM melatonin, 2.5% isopropanol, prepared from nuclease free H₂O and 100% isopropanol. Isopropanol is needed to fully solubilize melatonin. Store the melatonin solution at 4°C. The solution can be stored for at least two weeks.



Dye-Terminator Removal from DNA cycle sequencing reactions of PCR-products and Plasmids after use ABI Prism™ terminator Kits

Protocol 1B (384-well PCR plate, for 5-10 µL sequencing reaction mix)

1. Before use, shake MagSi-DNA clean^{FIX} particle mix to fully resuspend the beads.
2. Add 5 µL MagSi-DNA clean^{FIX} magnetic particle mix to each sample.
3. Add alcohol mix* (see Kit Contents, page 3) according to the table below; mix well by pipetting up and down 10 times.

Sequencing reaction volume (µL)	Volume of Alcohol mix (µL)
5	13
10	20

4. Incubate for 3 minutes at room temperature.
5. Place the sample plate on the magnetic separator for 3 minutes to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
6. **Washing of the magnetic beads:** Add 30 µL alcohol mix* and resuspend the magnetic beads by pipetting up and down 15 times.
7. Place the sample plate on a magnetic separator for 3 minutes to collect the magnetic beads completely. **Discard the supernatant.** Repeat steps 6-7 once more for a total of 2 washing steps.
8. Remove the samples from the magnet and air-dry the magnetic particles for 5 minutes.
9. **Elution of the DNA fragments:** Add 30 µL Elution Buffer to the magnetic bead sample and mix by pipetting up and down 10 times. Incubate for 2 minutes at room temperature.
10. Place the sample plate on a magnetic separator for 3 minutes to collect the magnetic beads completely. **Transfer the supernatant to a clean container, but leave ±5 µL liquid behind to prevent transfer of beads.**

Technical notes:

An elution volume of 30 µL is sufficient to ensure proper elution of the DNA fragments. A higher volume (up to 40 µL) can be used, but a minimum volume of 20 µL is needed to ensure contact between the liquid and the magnetic particles. For Elution buffer, reagent grade water or 0.1 mM EDTA can be used, depending on the preferred signal intensity.

For better stability of the purified sample, add mineral oil (Carl Roth, HP50.1) to the purified sample, optionally followed by short centrifugation to assure proper sealing.

Another possible optimization is to perform the elution in 2.25 mM melatonin, 2.5% isopropanol, prepared from nuclease free H₂O and 100% isopropanol. Isopropanol is needed to fully solubilize melatonin. Store the melatonin solution at 4°C. The solution can be stored for at least two weeks.



Purification and concentration of DNA fragments from enzymatic reactions, like products from PCR reactions, cDNA synthesis and enzyme restriction digestions

Protocol 2A (96-well PCR plate, for 10-20 μ L PCR product):

- 1. Before use, shake MagSi-DNA clean^{FIX} particle mix to fully resuspend the beads.**
- 2. Add 10 μ L MagSi-DNA clean^{FIX} magnetic particle mix to each sample**
- 3. Add MagSi-DNA clean Buffer P according to the table below; mix well by pipetting up and down 10 times.**

PCR Sample volume (μ L)	MagSi-DNA clean Buffer P (μ L)
10	20
20	30

- 4. Incubate for 3 minutes at room temperature**
- 5. Place the sample plate on the magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
- 6. Washing of the magnetic beads: Add 100 μ L EtOH 70%** and resuspend the magnetic beads by pipetting up and down 15 times.
- 7. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Repeat steps 6-7 once more for a total of 2 washing steps.
- 8. Remove the samples from the magnet and air-dry the magnetic particles for 5 minutes.**
- 9. Elution of the DNA fragments: Add 40 μ L Elution Buffer** to the magnetic bead sample and mix by pipetting up and down 10 times. Incubate for 2 minutes at room temperature.
- 10. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container, but leave ± 5 μ L liquid behind to prevent transfer of beads.**

Technical notes:

An elution volume of 40 μ L is sufficient to ensure proper elution of the DNA fragments. A higher volume (up to 100 μ L) can be used, but a minimum volume of 30 μ L is needed to ensure contact between the liquid and the magnetic particles. For Elution buffer, reagent grade water, TRIS-Acetate pH 8.0, TE-buffer can be used.



Purification and concentration of DNA fragments from enzymatic reactions, like products from PCR reactions, cDNA synthesis and enzyme restriction digestions

Protocol 2B (384-well PCR plate, for 5-10 μ L PCR product)

- 1. Before use, shake MagSi-DNA clean^{FIX} particle mix to fully resuspend the beads.**
- 2. Add 5 μ L MagSi-DNA clean^{FIX} magnetic particle mix to each sample**
- 3. Add MagSi-DNA clean Buffer P according to the table below; mix well by pipetting up and down 10 times.**

PCR sample volume (μL)	MagSi-DNA clean Buffer P (μL)
5	10
10	15

- 4. Incubate for 3 minutes at room temperature**
- 5. Place the sample plate on the magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
- 6. Washing of the magnetic beads: Add 30 μ L EtOH 70%** and resuspend the magnetic beads by pipetting up and down 15 times.
- 7. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Repeat steps 6-7 once more for a total of 2 washing steps.
- 8. Remove the samples from the magnet and air-dry the magnetic particles for 5 minutes.**
- 9. Elution of the DNA fragments: Add 30 μ L Elution Buffer** to the magnetic bead sample and mix by pipetting up and down 10 times. Incubate for 2 minutes at room temperature.
- 10. Place the sample plate on a magnetic separator for 3 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container, but leave \pm 5 μ L liquid behind to prevent transfer of beads.**

Technical notes:

An elution volume of 30 μ L is sufficient to ensure proper elution of the DNA fragments. A higher volume (up to 40 μ L) can be used, but a minimum volume of 20 μ L is needed to ensure contact between the liquid and the magnetic particles. For Elution buffer, reagent grade water, TRIS-Acetate pH 8.0, TE-buffer can be used.



Troubleshooting (Dye-Terminator Removal)

Problem	Probable cause	Suggestion
Dye Blobs (dye peaks usually at 70 and 100 bases)	Insufficient supernatant removal	Check the plate visually after discarding supernatant and wash solutions and make sure they are removed completely
	Too much BigDye	Use less BigDye per sequencing reaction
Low signal (signal intensity is similar to intensity of background noise)	Insufficient mixing	Make sure the elution volume is sufficient, the appropriate number of mixes are performed and visually check for proper homogenization
	Loss of magnetic particles	Make sure no magnetic particles are aspirated by proper positioning of the pipette, dispense back supernatant when aspiration of beads occurs
	Low alcohol concentration	Make sure the alcohol mixture is prepared freshly on the day of clean-up and correct volumes are added
Overload (signal intensity is extremely high)	Too much BigDye	Use less BigDye per sequencing reaction; transfer only part of the eluant for loading; use alternative elution buffer; decrease the sample injection time of the sequencer



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