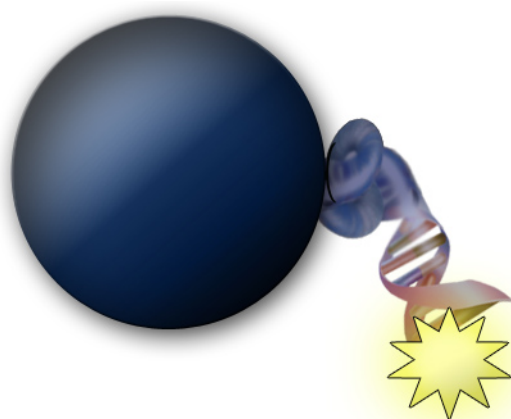




MagSi-DNA Clean Kit

Manual



Version 2.8 | 0710



MagSi-DNA clean kit

PCR Clean-up and Dye-Terminator Removal

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

Table of Contents

Table of Contents	2
General Information	2
Kit contents	3
Materials Supplied by the User	4
Kit usage	5
Protocol 1a	6
Protocol 1b	7
Protocol 2a	8
Protocol 2b	9
Troubleshooting	10

General information

The **MagSi-DNA clean kit** provides a convenient tool for ultra-fast and efficient direct purification of PCR products from 80 bp up to 30 kb from amplification reactions. The DNA fragments will be bound directly onto the surface of the magnetic beads. Finally, the DNA fragments will be eluted with low salt buffer or ddH₂O. 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.



MagSi-DNA clean kit contents

Contents	150 preparations	15.000 preparations
Article Number	MD60013	MD60014
MagnaMedics Dye Removal Reagent (M-DRR)	Reconstitute in 150 µl glycerol 50%	Reconstitute in 15 ml glycerol 50%
MagSi-DNA clean	600 µl magnetic particle mix	60 ml magnetic particle mix
Manual	1	1

Important:

Please aliquot the MagnaMedics Dye Removal Reagent (M-DRR) directly after delivery of the Kit. Store the aliquots at -20°C.

Resuspend the beads by vortexing intensively before use.



Materials Supplied by the User

Consumables & Equipment	
Multichannel pipettes	10 and 200 μ l
96/384-wells PCR plates, PCR tubes	suggested: Framestar®, 4titude® Ltd, Cat. No. 4ti-0384/C
Magnetic separators	Suitable for 384-wells PCR plate (total working volume 40-120 μ l/well) 96-wells MTP format (total working volume 200 μ l/well), or 200 μ l PCR tubes
Thermoblock	Up to 45-50°C

Reagents	
Isopropanol p.a. (suggested: VWR p/n 1.00983.1011)	10 ml
Pure Ethanol p.a. (suggested: VWR p/n 1.00013.1000)	0.25 ml
Elution buffer	ddH ₂ O or standard TE buffer

- **Alcohol mix*: 95% isopropanol (p.a.) and 5% Ethanol (p.a) to be prepared by the user. Single purifications for 10 and 20 μ l sample volumes require respectively 26, and 90 μ l Alcohol mix.**



Kit usage

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

Protocols	PCR plate format	Total working volume	Total sample volume
1a and 2a	384 well	40,5 µl	10 µl sample mix
1b and 2b	96 well and manual	120 µl	20 µl sample mix

Preparation time is approx. 20 - 35 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 under each MagnaMedics kit and kit component.

Further equipment/disposables supplied by the client:

- **Disposable gloves**
- **Pipettes and pipette tips**
- **Vortex**
- **Reaction tubes (1.5 ml or 2.0 ml)**



Protocol 1a:

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

protocol for sample volume up to 10 μ l (384-well PCR plate)

- 1. Binding of the PCR-fragments:** To 10 μ l PCR mix add first 26 μ l alcohol mix* (see Kit Contents) and secondly add 4 μ l MagSi-DNA clean particle mix; mix well by pipetting up and down 10 times.
- 2. Incubate for 3 minutes at room temperature**
- 3. Place the 384-wells PCR plate on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
- 4. Washing of the magnetic beads:** Add 40 μ l alcohol mix* and resuspend the magnetic beads by pipetting up and down 10 times. **Incubate for 1 minute at room temperature (RT) and place the 384-wells PCR plate on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Air-dry the magnetic beads for 1 minute.
- 5. Elution of the PCR fragments:** Add 15-25 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.
- 6. Place the 384-wells PCR plate on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container to perform the sequencing reaction.** Try to avoid pipetting magnetic particles as the liquid contains purified sample free of contaminants.

Technical note: For better stability of the purified sample, the elution can be performed 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. Another possible optimization for better stability is the addition of 15 μ l mineral oil to the purified sample, followed by short centrifugation to assure proper sealing.



Protocol 1b:

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

protocol for sample volume up to 20 μ l (microtube or 96-well plate)

- 1. Binding of the PCR-fragments:** To 20 μ l PCR mix add first 90 μ l alcohol mix* (see Kit Contents) and secondly add 4 μ l MagSi-DNA clean particle mix; mix well by pipetting up and down 10 times.
- 2. Incubate for 3 minutes at room temperature**
- 3. Place the samples on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Make sure you do not remove any of the magnetic beads.
- 4. Washing of the magnetic beads:** add 120 μ l alcohol mix* and resuspend the magnetic beads by pipetting up and down 10 times. **Incubate for 1 minute at room temperature (RT) and place the samples on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Air-dry the magnetic beads for 1 minute.
- 5. Elution of the PCR fragments:** Add 15-25 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.
- 6. Place the samples on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container to perform the sequencing reaction.** Try to avoid pipetting magnetic particles as the liquid contains purified sample free of contaminants.

Technical note: For better stability of the purified sample, the elution can be performed 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. Another possible optimization for better stability is the addition of 15 μ l mineral oil to the purified sample, followed by short centrifugation to assure proper sealing.



Protocol 2a:

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

protocol for sample volume up to 10 µl (384-well PCR plate)

Dye blob reduction: To reduce any dye blobs in the DNA sequencing read out perform an upfront dye blob reduction step (step 1-2) prior to the DyeDeoxy removal itself. Depending on the final specifications in terms of DNA sequencing, read-out length and sequencing quality this step might be prolonged or additional dye removal reagent (DRR) might be added.

- 1. To 10 µl sequencing reaction, add 1 µl DRR. Mix well.**
- 2. Incubate the mixture 5-10 minutes at room temperature.** For high performance DNA sequencing results, the incubation time can be prolonged to 15-30 min. In addition, the incubation time has to be adapted to the individual liquid handling setup.
- 3. Binding of the DNA fragments: First add 25,5 µl alcohol mix* (see Kit Contents) and secondly add 4 µl MagSi-DNA clean particle mix;** mix well by pipetting up and down 10 times.
- 4. Incubate for 3 minutes at room temperature**
- 5. Place the 384-wells PCR plate on a magnetic separator for 2 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 40 µl alcohol mix*** and resuspend the magnetic beads by pipetting up and down 10 times. **Incubate for 1 minute at room temperature (RT) and place the 384-wells PCR plate on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Air-dry the magnetic beads for 1 minute.
- 7. Elution of the DNA fragments: Add 15-25 µl Elution Buffer** to the magnetic bead sample. Mix by pipetting up and down 5 times. Incubate for 2 minutes at room temperature.
- 8. Place the 384-wells PCR plate on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container.** Try to avoid pipetting magnetic particles as the liquid contains purified sample free of contaminants.

Technical note: For better stability of the purified sample, the elution can be performed 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. Another possible optimization for better stability is the addition of 15 µl mineral oil to the purified sample, followed by short centrifugation to assure proper sealing.



Protocol 2b:

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

protocol for sample volume up to 20 µl (96-well plate or microtube)

Dye blob reduction: To reduce any dye blobs in the DNA sequencing read out perform an upfront dye blob reduction step (step 1-2) prior to the DyeDeoxy removal itself. Depending on the final specifications in terms of DNA sequencing, read-out length and sequencing quality this step might be prolonged or additional dye removal reagent (DRR) might be added.

- 1. To 20 µl sequencing reaction, add 2 µl DRR. Mix well.**
- 2. Incubate the mixture 5-10 minutes at room temperature.** For high performance DNA sequencing results, the incubation time can be prolonged to 15-30 min. In addition, the incubation time has to be adapted to the individual liquid handling setup.
- 3. Binding of the DNA fragments: First add 90 µl alcohol mix* (see Kit Contents) and secondly add 4 µl MagSi-DNA clean particle mix;** mix well by pipetting up and down 10 times.
- 4. Incubate for 3 minutes at room temperature**
- 5. Place the samples on a magnetic separator for 2 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 120 µl alcohol mix*** and resuspend the magnetic beads by pipetting up and down 10 times. **Incubate for 1 minute at room temperature (RT) and place the samples on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Discard the supernatant.** Air-dry the magnetic beads for 1 minute.
- 7. Elution of the DNA fragments: Add 15-25 µl Elution Buffer** to the magnetic bead sample. Mix by pipetting up and down 5 times. Incubate for 2 minutes at room temperature.
- 8. Place the samples on a magnetic separator for 2 minutes** to collect the magnetic beads completely. **Transfer the supernatant to a clean container.** Try to avoid pipetting magnetic particles as the liquid contains purified sample free of contaminants.

Technical note: For better stability of the purified sample, the elution can be performed 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. Another possible optimization for better stability is the addition of 15 µl mineral oil to the purified sample, followed by short centrifugation to assure proper sealing.



Troubleshooting

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 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

MagnaMedics Diagnostics B.V.

**IVD, Food-Diagnostics, Life-Science Products,
Laboratory & Production**

Chemelot Campus

Burgemeester Lemmenstraat 366

6163 JT Geleen (The Netherlands)

Manual_MagSi-DNA_clean_ver2.8_1207.odt



Tel: +31-(0)46-8200206

Fax: +31-(0)46-4106825

E-mail: info@magnamedics.com

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