magtivio

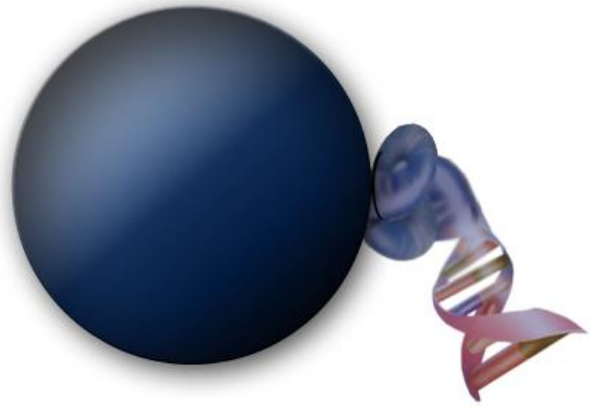
MagSi-DT Removal

Art.No.

MDKT00040008

MDKT00040050

MDKT00040500



Product Manual

Version 3.1 | 12-12-2022

Revision history		
Revision	**Release date**	**Remarks**
1.0	07/08/2018	Initial release
3.0	02/06/2022	Layout changes, revision history added
3.1	12/12/2022	New company style

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1. General Information

1.1 Intended Use

MagSi-DT Removal is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

MagSi-DT Removal provides a convenient tool for ultra-fast and efficient purification of Dye-Terminator sequencing reactions and removal of unincorporated Dye Terminators and salts. The kit can be used manually or on automated workstations using 96- and 384-well PCR plates.

1.2 Kit specifications

The number of preparations depends on the plate format. Please refer to the table below to determine how many purifications can be performed.

	MDKT00040008 **8 mL**	**MDKT00040050** **50 mL**	**MDKT00040500** **500 mL**
96 Well Format	800	5.000	50.000
384 Well Format	1600	10.000	100.000

1.3 Basic principle

MagSi-DT Removal uses a simple three-step protocol and it can be used directly in the thermal cycling plate. MagSi-DT Removal beads are added to the sequence reaction product. Binding conditions are adjusted by adding ethanol 85%. DNA binds to the magnetic beads while unincorporated dye terminators and salts in solution. The beads are washed twice in 85% ethanol to remove traces of interfering components. The DNA fragments are eluted with reagent grade water or 0.1 mM EDTA pH 8.0. The total preparation time is approximately 20 minutes and hands-on time necessary for the whole procedure is reduced to a minimum.

2. Materials

2.1 Kit Contents

Article Number	MDKT00040008	MDKT00040075	MDKT00040500
Kit size	8 mL	50 mL	500 mL
Product Manual	1	1	1

2.2 Reagents, consumables and equipment to be supplied by the user

Reagents	
Ethanol p.a.	VWR cat# 1.00013.1000
Elution buffer	Reagent grade water or 0.1 mM EDTA pH 8.0

Consumables & Equipment	
Multichannel pipettes	10 µL and 100 µL
Reaction plate	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 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

3. Kit usage

3.1 Storage Conditions

The kit should be stored at 2-8°C. When stored under the conditions mentioned, the kit is stable for up to 2 years, but no longer than the expiry date on the label. Do not freeze!

3.2 Preparation of reagents

- Prepare ethanol solution: Make 25 mL of 85% ethanol per 96 well plate. Ethanol is used for precipitation of DNA products, so it is critical that the 85% ethanol has been prepared fresh.
- Prepare Elution buffer: Reagent grade water or 0.1 mM EDTA pH 8.0
- Shake MagSi-DT Removal to fully resuspend the magnetic beads. The reagent should be homogeneous and consistent in color.

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 the MagSi-DT Removal 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.

3.4 Considerations

1. Drying time may vary due to differences in the laboratory environment. Careful: Do not over-dry the samples, this may result in loss of signal intensity.
2. When transferring purified samples, leave a small volume of liquid behind in order to prevent carry-over of magnetic beads into a new plate. Residual magnetic beads can interfere with injection. If beads are present in final sample, perform the separation again.
3. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.
4. Process only as many samples in parallel as the magnetic separator allows.

3.5 Magnetic Separation systems

MagSi-DT Removal has been designed for manual and automated use. For processing in 96-well and 384-well PCR plates we recommend to use the MM-Separator 96 PCR (Art.No. MDMG0005) and MM-Separator 384 PCR (Art.No. MDMG0006).

For use with other magnetic separators, please contact the technical support at support@magtivio.com.

3.6 Product use limitations

MagSi-DT Removal is intended for Research Use Only. Do not use for other purposes than intended. The kit components can be used only once.

The end-user has to validate the performance of the kit for any particular use, since the performance characteristics of the kits have not been validated for any specific application. magtivio kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

The product is intended for use by trained personnel. Diagnostic results generated using the sample preparation procedure should only be interpreted with regard to other clinical or laboratory findings. Adequate controls should be used in each set of isolations, especially when used for diagnostic purposes.

4. Protocols

4.1 Dye-Terminator Removal in 96 well plate

1. Before use, shake MagSi-DT Removal to fully resuspend the beads.
2. Add 10 µL MagSi-DT Removal to each sample in the plate.
3. Add 85% ethanol according to the table below. Mix by pipetting up and down 10 times until a homogeneous suspension is obtained. It is very important to mix to completely bind the sequencing products.

Sequencing reaction volume (µL)	Volume of 85% ethanol (µL)
5	31
10	42
15	52
20	62
25	73

For other volumes use this calculation: Volume of 85% ethanol = 2.077 x (10 µL + Sample Volume)

4. Place the sample plate on the magnetic separator for 3 minutes to collect the magnetic beads until a clear solution is obtained.
5. 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.
6. Add 100 µL of 85% ethanol 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)
7. Completely discard the supernatant. This step must be performed while the plate is on the magnet.
8. Repeat steps 6-7 once more for a total of 2 washing steps.
9. Air-dry the magnetic beads for 10 minutes. The beads can be dried while the plate is on the magnet. Note: Excessive drying can lead to degradation of the fluorescent dye.
10. Add 40 µL Elution Buffer and incubate for 5 minutes to elute. This step can be performed while the plate is placed on the magnet. Elution is fast but can be facilitated by pipetting up and down 7 times.
11. Transfer the supernatant to the final plate. Leave 5-10 µL liquid behind to prevent transfer of beads into the final plate.

4.2 Dye-Terminator Removal in 384 well plate

1. Before use, shake MagSi-DT Removal to fully resuspend the beads.
2. Add 5 µL MagSi-DT Removal to each sample in the plate.
3. Add 85% ethanol according to the table below. Mix by pipetting up and down 10 times until a homogeneous suspension is obtained. It is very important to mix to completely bind the sequencing products.

Sequencing reaction volume (µL)	Volume of 85% ethanol (µL)
5	14.3
10	21.4
15	28.6

For other volumes use this calculation: Volume of 85% ethanol = 1.428 x (5 µL + Sample Volume)

4. Place the sample plate on the magnetic separator for 2-3 minutes to collect the magnetic beads until a clear solution is obtained.
5. 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.
6. Add 30 µL of 85% ethanol 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)
7. Completely discard the supernatant. This step must be performed while the plate is on the magnet.
8. Repeat steps 6-7 once more for a total of 2 washing steps.
9. Air-dry the magnetic beads for 10 minutes. The beads can be dried while the plate is on the magnet. Note: Excessive drying can lead to degradation of the fluorescent dye.
10. Add 15-30 µL Elution Buffer and incubate for 5 minutes to elute. This step can be performed while the plate is placed on the magnet. Elution is fast but can be facilitated by pipetting up and down 7 times.
11. Transfer the supernatant to the final plate. Leave 2-5 µL liquid behind to prevent transfer of beads into the final plate.

5. 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 the elution volume is sufficient, the appropriate number of mixes are performed and visually check for proper homogenization - Optionally resuspend the sample 1-2 times during binding step.
	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 ethanol concentration	- Make sure 85% ethanol 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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