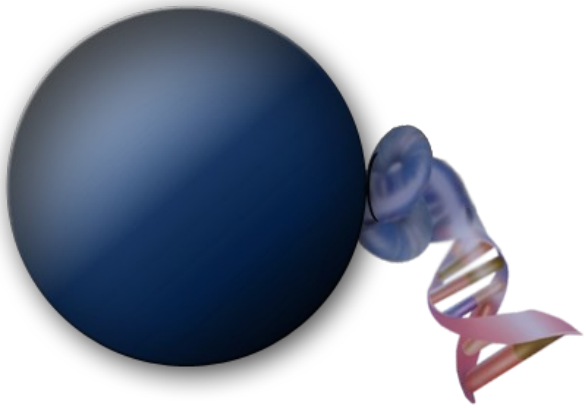
magtivio

MagSi-DT Removal

Art.No.
MDKT0004



Product Manual

Version 2.0 | 07/08/2018

MagSi-DT Removal

Dye-Terminator Removal

This product is for Research Use Only (RUO). Not for drug, household or other uses. For more information, please consult the appropriate Safety Data Sheet (SDS), available on our website at www.magtivio.com

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1. General Information

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. The kit uses a simple three-step protocol and it can be used directly in the thermal cycling plate. Dye labeled DNA products are bound onto the surface of the magnetic beads, leaving unincorporated Dye Terminators and salts in solution. 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.

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

Table 1. Number of preparations for different kits sizes

	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

2. Materials supplied in the kit

Table 2. Materials supplied for different kit sizes

Art.No.	MDKT00040008	MDKT00040050	MDKT00040500
MagSi-DT Removal beads	8 mL	50 mL	500 mL
Product manual	1	1	1

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

2.1 Materials Supplied by the User

Table 3. Materials supplied by the user

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 (Magtivio, Art.No.: MDMG0005): Magnetic separator for 96-well PCR plates, U and V-bottom microplates. suitable for automated processes MM-Separator 384 PCR (Magtivio, Art.No.: MDMG0006): Magnetic separator for 384-well PCR. suitable for automated processes
Reagents	
Ethanol p.a.	VWR cat# 1.00013.1000
Elution buffer	Reagent grade water or 0.1 mM EDTA pH 8.0

3. Kit Usage

3.1 Preparations before use

- 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.

3.2 Handling guidelines

- 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.
- 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.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDS).

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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