Steinbrenner Laborsysteme GmbH

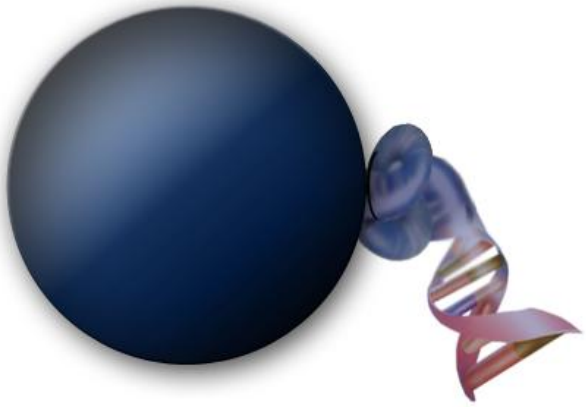
magtivio

MagSi-DNA Tissue & Cells

Art.No.

MDKT00180096

MDKT00180960



Product Manual

Version 2.1 | 07/12/2022

Revision history		
Revision	**Release date**	**Remarks**
1.0	26/08/2021	Initial release
2.0	08/03/2022	Updated section 4.3, layout changes
2.1	07/12/2022	New company style

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1. General Information

1.1 Intended Use

MagSi-DNA Tissue & Cells is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

The kit is intended for manual and automated isolation of genomic DNA from mammalian tissue samples and eukaryotic cells. Processing time for the preparation of 96 samples is about 40 minutes plus an additional pre-lysis incubation of 15 min (cells), 1-3h or overnight for tissue samples. The kit requires no phenol/chloroform extraction or alcohol precipitation and eliminates the need for repeated centrifugation, vacuum filtration or column separation. It allows safe handling of potentially infectious samples, and is designed to avoid sample-to-sample cross-contaminations. The obtained DNA can be used directly for downstream applications such as PCR, or any kind of enzymatic reaction.

MagSi-DNA Tissue & Cells is suitable for use with fresh or frozen tissue samples or eukaryotic cell pellets. It is possible to extract DNA from bacteria but this may require additional pre-treatment (lytic enzymes or mechanical disruption which are not included in the kit).

MagSi-DNA Tissue & Cells beads are optimized for use in isolating total DNA. The beads are easy to handle and are supplied in an optimized storage buffer for increased suspension time. Depending on the sample materials RNA may be co-purified. If required RNase treatment has to be integrated in the purification protocol. RNase is not included in the kit.

Following the lysis incubation at 56°C all other steps of the procedure can be processed at room temperature. However, elution at 72°C may increase yields slightly.

1.2 Kit specifications

The kit provides reagents for extraction of 10–20 µg DNA from up to 20 mg tissue samples or up to 1 x 106 cells samples with an A260/A280 ratio of >1.7 and A260/A230 ratio of >1.5, with typical concentrations of 50-100 ng/µL. Depending on the elution volume used, concentrations of up to 200 ng/µL can be obtained.

The DNA obtained can be stored at 2-8°C. For long-term use, storage at -20°C is recommended. To maintain the high-molecular weight nature of the isolated DNA, it is recommended to avoid multiple freeze-thaw cycles.

1.3 Basic principle

Tissue and cells samples are lysed under denaturing conditions by adding Lysis Buffer TS and Proteinase K at 56°C. After lysis incubation, remaining debris are removed by centrifugation. MagSi-TC VI beads are added to the cleared supernatant and binding conditions are adjusted by addition of Binding Buffer U1 so that DNA binds to the magnetic beads. After magnetic separation and discard of the supernatant, the beads are washed three times to remove contaminants and salts. A drying step makes sure all traces of ethanol are removed. Finally, purified DNA is eluted with low-salt elution buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	96 preps MDKT00180096	10 x 96 preps MDKT00180960
Lysis Buffer TS	25 mL	250 mL
Binding Buffer U1	40 mL	400 mL
Proteinase K	20 mg (for 1.1 mL working solution)	200 mg (for 11 mL working solution)
MagSi-TC VI	2 mL	20 mL
Wash Buffer I	2 x 80 mL	2 x 800 mL
Wash Buffer II	80 mL	800 mL
Elution Buffer	20 ml	200 mL
Manual	1	1

For DNA extraction protocols without a drying step (Protocol 4.2), Wash Buffer III can be ordered separately (Art.No. MD70041). Please contact magtivio for further information.

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

2.2.1 Reagents

- molecular biology grade (nuclease free) water to reconstitute Proteinase K
- If RNA free DNA is required: RNase A solution 20 mg/mL, 20 µL per sample

2.2.2 Consumables and equipment for manual use or automated processing on liquid handling robots

Protocol	Manual use	Automated use
Sample containers	1.5 or 2 mL microtubes	Recommended: Riplate®SW 96, PP, 2ml, (Ritter, 43001–0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0mL, (Thermo Scientific, Cat.No. 278752)
Magnetic separation	MM-Separator M12 + 12 P Art.No. MDMG0001	MM-Separator 96 DeepWell Art.No. MDMG0013
Final container	1.5 or 2 mL microtubes	96-well microplate
Lysis / mixing	Heater shaker for tubes / tube Vortexer	Heater microplate shaker (min. 1000 RPM)

2.2.3 Consumables for processing on the PurePrep 96 System or KingFisher™ Flex instrument

Product	Art. No.	Contents
2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	MDPL00200060	60 pieces
200 µL square-well Elution Plate for KingFisher™/PurePrep 96	MDPL00190060	60 pieces
96 well Tip-Comb for KingFisher™/PurePrep 96	MDPL00210060	60 pieces

3. Kit usage

3.1 Storage Conditions

All components of the kit should be stored at room temperature (18-25°C). Store working solutions of reconstituted Proteinase K at -20°C. When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Preparation of reagents

- Reconstitute Proteinase K:
- MDKT00180096 (96 preps), add **1.1 mL of diH2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C
- MDKT00180960 (10x96 preps), add **11 mL of diH2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C
- If there is any precipitate present in the buffers, warm the buffer to 25-37°C to dissolve the precipitate before use.
- Immediately before use, resuspend MagSi-TC VI beads by vortexing for 20 seconds. If preferred, MagSi-TC VI beads can be premixed with Binding Buffer U1 for simultaneous addition to samples. The mixture must used on the day of preparation, and mixed well by vortexing before transfer to samples. For each sample, prepare Binding Buffer / Beads premix:

Binding Buffer U1	400 µL
MagSi-TC VI	20 µL
Total	420 µL

- Samples should be thoroughly mixed before aliquotation

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-DNA Tissue and Cells kit 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. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.
2. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.
3. Do not combine components of different kits unless the lot numbers are identical.
4. Process only as many samples in parallel as the magnetic separator allows.
5. The elution can be done in smaller volumes of Elution Buffer. Although this may result in higher DNA concentrations, overall yield may be lower. The yield may also be increased by prolonging the incubation time, and with pre-heated Elution Buffer (72°C).
6. The Elution Buffer does not contain EDTA (the end user may wish to use other elution buffers containing EDTA, or Tris and EDTA, though).
7. The kit is intended to be used for up to 20 mg of tissue samples or up to 1x106 cells. Exceeding the sample amount may result in lower DNA yield due to incomplete bead separations out of viscous sample lysates or due to low purity.
8. A complementary wash buffer for MagSi-DNA Tissue & Cells to replace the drying step can be ordered separately, Wash Buffer III (WB3), Art.No. MD70041. Wash Buffer III eliminates risks of inhibition by residual alcohols, and may increase DNA purity.
9. It may occur that a small amount of beads is accidentality transferred with the final DNA sample, but most likely this will not inhibit subsequent applications. However, if desired another separation step can be performed to remove the beads.

3.5 Magnetic Separation systems

MagSi-DNA Tissue & Cells has been designed for use on the MM-Separator 96 DeepWell and MM-Separator M12 + 12 P. The MM-Separator M12 + 12 P (Art.No. MDMG0001) allows simultaneous processing of up to 12 samples in 2 mL microcentrifuge tubes. For processing in 96 deepwell plates, use the MM-Separator 96 DeepWell (Art.No. MDMG0013).

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

MagSi-DNA Tissue & Cells is compatible with the PurePrep 96 System and the KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Information of use on these instruments is described in sections 4.3 and 4.4 . Software protocol files are available on request.

3.6 Shaker settings

The speed settings for the microplate shaker described in the protocols that follow were defined with a specific instrument and microplate. When first using a plate shaker for incubation steps, the speed settings have to be set carefully for each specific plate to prevent cross contamination and spillage. Setting the speed of the shaker can be done by loading a microplate with a volume of dyed water equal to the working volume during each step, and step-wise increasing the shaker speed until droplets are observed on the surface of the plate. Set the shaker speed lower again.

3.7 Product use limitations

MagSi-DNA Tissue & Cells is intended for Research Use Only. Do not use for other purposes than intended. The kit components can be used only once.

No guarantee is offered when using sample material other than human or animal tissue samples or cultured eukaryotic cells. The kit is not validated for isolating DNA from for instance stool, bacteria, fungi or viruses, and is also not validated for the isolation of RNA.

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. The isolated DNA can be used in most genomic applications, such as restriction digestion, PCR, sequencing.

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 Manual DNA extraction (with drying step)

Before starting

- Check if Proteinase K was prepared according to section 3.2.
- Vortex magnetic beads thoroughly into a homogeneous suspension.

1. Transfer the samples into a 96-deepwell microplate or microtubes.
2. Add **225 µL Lysis Buffer TS** and **10 µL Proteinase K.** Incubate tissue samples for at least 1 h or overnight under moderate shaking at 56°C. The sample should be disintegrated after the lysis incubation. Make sure that the tissue samples are submerged into the lysis buffer during lysis incubation. Cell samples typically require a lysis incubation of 15 min.

   If RNA free DNA is required: Add 20 µL of a 20 mg/mL RNase A solution (not supplied with the kit) after the lysis incubation. Incubate with shaking for 10 min at room temperature.
3. Centrifuge the lysed sample for 5 min at full speed to collect any unlysed material to the bottom of the container used for processing. Transfer 200 µL of the cleared lysate to a new container. Avoid transfer of undigested sample material or debris.
4. Add **400 µL Binding Buffer U1** and **20 µL MagSi-TC VI.** Incubate on a shaker for 5 min at 1000 RPM.
5. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** to the tubes. Incubate on a shaker for 1 min at 1000 RPM. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Repeat step 5 one more time with **800 µL Wash Buffer I** and one time with **800 µL Wash Buffer II**
8. Dry the beads on air for 5 min to evaporate the ethanol completely.
9. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer** . Incubate on a shaker for 5 min at 1000 RPM.
10. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.
    - If the transferred eluates appear turbid, briefly centrifuge the samples and carefully transfer the eluates.
    - If the transferred eluates contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates.
    - Elution at 72°C may increase yields.

- The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.

4.2 Manual DNA extraction with Wash Buffer III*

*Wash Buffer III (Art.No. MD70041) needs to be ordered separately.

Before starting:

- Check if Proteinase K was prepared according to section 3.2.
- Vortex magnetic beads thoroughly into a homogeneous suspension.

1. Transfer the samples into a 96-deepwell microplate or microtubes.
2. Add **225 µL Lysis Buffer TS** and **10 µL Proteinase K.** Incubate tissue samples for at least 1 h or overnight under moderate shaking at 56°C. The sample should be disintegrated after the lysis incubation. Make sure that the tissue samples are submerged into the lysis buffer during lysis incubation. Cell samples typically require a lysis incubation of 15 min.

   If RNA free DNA is required: Add 20 µL of a 20 mg/mL RNase A solution (not supplied with the kit) after the lysis incubation. Incubate with shaking for 10 min at room temperature
3. Centrifuge the lysed sample for 5 min at full speed to collect any unlysed samples to the bottom of the container used for processing. Transfer 200 µL of the cleared lysate to a new container. Avoid transfer of undigested sample material or debris.
4. Add **400 µL Binding Buffer U1** and **20 µL MagSi-TC VI.** Incubate on a shaker for 5 min at 1000 RPM.
5. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** to the tubes. Incubate on a shaker for 1 min at 1000 RPM. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Repeat step 5 one more time with **800 µL Wash Buffer I** and one time with **800 µL Wash Buffer II**
8. With the samples on the magnet, slowly add **800 µL Wash Buffer III**. Wait for 30 seconds and carefully remove the supernatant again. Do not resuspend beads and do not exceed 60 seconds as this may cause early DNA elution. When using the kit manually, it is recommended to not treat samples with Wash Buffer III simultaneously.
9. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer** . Incubate on a shaker for 5 min at 1000 RPM.
10. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.
    - If the transferred eluates appear turbid, briefly centrifuge the samples and carefully transfer the eluates.
    - If the transferred eluates contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates.

- Elution at 72°C may increase yields.
- The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.

4.3 Protocol for the PurePrep 96 System

4.3.1 PurePrep 96 software protocol file

Please contact magtivio for the most recent software method files. We provide the corresponding files for direct upload on the PurePrep 96 System. Refer to the PurePrep 96 user manual regarding the upload procedure of the supplied software files to the instrument.

4.3.2 Sample lysis (offline)

1. Transfer the samples into a 96-deepwell microplate.

2. Add **225 µL Lysis Buffer TS** and **10 µL Proteinase K.** Incubate tissue samples for at least 1 h or overnight under moderate shaking at 56°C. The sample should be disintegrated after the lysis incubation. Make sure that the tissue samples are submerged into the lysis buffer during lysis incubation. Cell samples typically require a lysis incubation of 15 min.

3. Centrifuge the lysed sample for 5 min at full speed to collect any unlysed samples to the bottom of the container used for processing. Transfer 200 µL of the cleared lysate to a 2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96. Avoid transfer of undigested sample material or debris.

4.3.3 Preparation of processing plates

Plate filling instructions

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Empty, for loading Tip-Comb only	N/A	1
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Lysate MagSi-TC VI beads Binding Buffer U1	200 µL 20 µL 400 µL	2
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	3
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	4
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL	5
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	150 µL	8

Suitable plates can be purchased at magtivio (see section 2.2). We strongly recommend using only the plates which are intended to use on the PurePrep 96 System. Using unsuitable plates may result in extraction failure or instrument damage.

4.3.4 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Prepare the “Sample Plate” for the binding step with MagSi-TC VI and Binding Buffer U1. To each well of the Sample Plate already containing 200 µL lysate, dispense **20 µL MagSi-TC VI** magnetic beads and **400 µL Binding Buffer U1** 🟢
2. Prepare “Wash Plate 1” and “Wash Plate 2” with **Wash Buffer I.** Add **800 µL Wash Buffer I** 🔵 to each well of the corresponding deep-well plates.
3. Prepare “Wash Plate 3” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
4. Prepare “Elution Plate” with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
5. Switch on the PurePrep 96 System and select the protocol from the user defined protocols
6. Load all plates to the PurePrep 96 instrument on indicated positions, see section 4.3.3 (right-most column). Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions.

   Make sure that the plates are loaded in the correct orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable. Make sure that the plates are fixed to the positions by the clamps.
7. Press on the Tab “Run Prog.”, select the shortcut icon for the protocol and press Run to start the protocol
8. At the end of the run remove all plates from the instrument

4.4 Protocol for the KingFisher Flex™

4.4.1 KingFisher BindIt sofware protocol

Please contact magtivio for the most recent BindIt software method files. We provide the corresponding files for direct upload on the KingFisher magnetic particle processors. A PDF description of the method file is included. Refer to the BindIt software manual regarding the upload procedure of the supplied software files to the instrument.

4.4.2 Sample lysis (offline)

1. Transfer the samples into a 96-deepwell microplate.
2. Add **225 µL Lysis Buffer TS** and **10 µL Proteinase K.** Incubate tissue samples for at least 1 h or overnight under moderate shaking at 56°C. The sample should be disintegrated after the lysis incubation. Make sure that the tissue samples are submerged into the lysis buffer during lysis incubation. Cell samples typically require a lysis incubation of 15 min.
3. Centrifuge the lysed sample for 5 min at full speed to collect any unlysed samples to the bottom of the container used for processing. Transfer 200 µL of the cleared lysate to a 2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96. Avoid transfer of undigested sample material or debris.

4.4.3 Preparation of processing plates

Plate filling instructions

Plate name	Plate type	Reagent (Kit component)	Volume
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Lysate MagSi-TC VI beads Binding Buffer U1	200 µL 20 µL 400 µL
Wash Plate 1	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 2	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 3	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	150 µL
Tip plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Empty, for loading Tip-Comb only	N/A

Suitable plates can be purchased at magtivio (see section 2.2). We strongly recommend using only the plates which are intended to use on the PurePrep 96 System. Using unsuitable plates may result in extraction failure or instrument damage.

4.4.4 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Prepare the “Sample Plate” for the binding step with **MagSi-TC VI** and **Binding Buffer U1.** To each well of the Sample Plate already containing 200 µL lysate, dispense **20 µL MagSi-TC VI** magnetic beads and **400 µL Binding Buffer U1** 🟢
2. Prepare “Wash Plate 1” and “Wash Plate 2” with **Wash Buffer I.** Add **800 µL Wash Buffer I** 🔵 to each well of the corresponding deep-well plates.
3. Prepare “Wash Plate 3” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
4. Prepare “Elution Plate” with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
5. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols
6. Start the protocol.
7. Load the plates to the instrument, following the instructions on the instrument display. Order of plates start with the tip plate and ends with the sample plate. The purification process starts immediately after loading the sample plate to the instrument.

   Make sure that the plates are loaded in the correct orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable.
8. At the end of the method remove all plates from the instrument. Follow the instructions on the instrument display.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	To much sample material	- Try lower sample input amount, avoid high sample amounts in combination with low elution buffer volumes
	Incomplete lysis	- Increase incubation time for lysis - Make sure that the sample is submerged in lysis buffer. If possible use heater shaker for optimal lysis. - Poor sample quality
	Inefficient binding to the magnetic particles	- Make sure Lysis Buffer TS and Binding Buffer U1 do not contain precipitates - Use correct amount of all reagents - Increase mixing steps after adding Binding Buffer U1 - Mix sample during binding incubation
	Incomplete elution	- Drying of Wash Buffer II may have been incomplete - Try eluting twice with 100 µL Elution Buffer
Degraded or sheared DNA	Incorrect storage of the sample material	- Sample should be collected and stored properly - Avoid repeated freezing and thawing of tissue or cell samples
Purified DNA samples are turbid	Sample lysates contains solid particulates	- Centrifuge briefly after lysis incubation and transfer samples to a new container
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Remove remaining traces from Wash Buffer II completely. Increase the drying time to 15 minutes - Use protocol with Wash Buffer III (see section 4.2)
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that supernatants are properly removed. - Wash Buffers should be stored and used at RT
	Magnetic beads remaining in the eluate	- Place the DNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

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Ordering Information

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4. End user name and telephone number (if different)
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