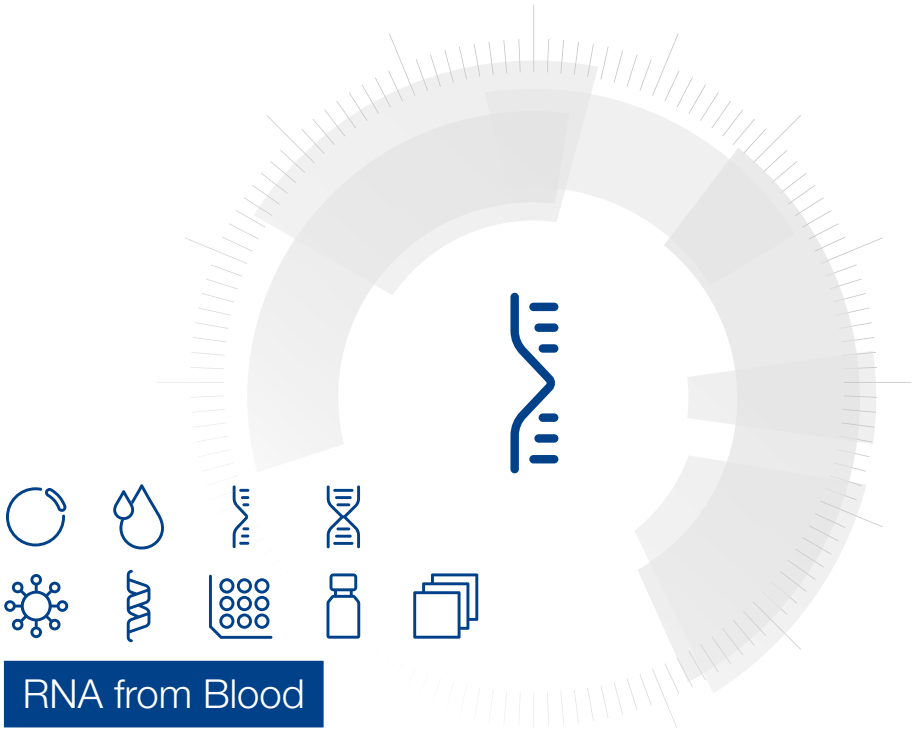
Bioanalysis

MACHEREY-NAGEL

User manual



RNA from Blood

■ NucleoMag® RNA Blood

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1 Components

1.1 Kit contents

	NucleoMag® RNA Blood	
REF	**1 × 96 preps 744352.1**	**4 × 96 preps 744352.4**
NucleoMag® B-Beads	1.8 mL	4 × 1.8 mL
Binding Buffer MRB1	50 mL	200 mL
Wash Buffer MRB2	125 mL	1 × 300 mL
		1 × 125 mL
RNase-free H_2O	30 mL	60 mL
Liquid Proteinase K	1.8 mL	7 mL
rDNase, lyophilized*	3 vials (size D)	12 vials (size D)
Reaction Buffer for rDNase	60 mL	3 × 60 mL
Leaflet	1	1

1.2 Reagents, equipment and consumables to be supplied by user

Reagents

- 80 % ethanol (v/v) for washing steps (absolute or non-denatured ethanol)

Samples

- Blood samples, collected in either EDTA or citrate blood collection tubes or in one of the following blood collection tubes (the blood collection tubes are not provided with the NucleoMag® RNA Blood kit):

Blood collection tube	Supplier
S-Monovette® RNA Exact	SARSTEDT (01.2048.001)
Tempus™ Blood RNA Tubes	Applied Biosystems by Thermo Fisher Scientific (4342792)
DNA/RNA Shield™ Blood Tubes	Zymo Research (R1150)

* For preparation of working solutions and storage conditions, see section 3.

Consumables

- Sterile RNase-free pipette tips (recommended)

Product	REF	Pack of
Magnetic separation system e.g., NucleoMag® SEP (see section 2.3); alternatively, liquid handling devices or automated magnetic rod extractors such as IsoPure™ Mini, MagnetaPure 32 Plus or KingFisher® Flex can be used*	744900	1
Separation plate for magnetic beads separation, e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24
For use of kit on KingFisher® Flex instrument: e.g., 96-well Acessory Kit B for KingFisher™ (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag® RNA preps using KingFisher® Flex platform)	744951	1 set
96 Deep-well plates for magnetic rod systems (e.g. for MagnetaPure 32 Plus or IsoPure™ Mini)	744955	25
8-Place tip compbs for magnetic rod systems (e.g. for MagnetaPure 32 Plus or IsoPure™ Mini)	744960	50

1.3 Additional buffers, consumables and equipment to be supplied for processing of Tempus™ Blood RNA Tubes

- Lysis Buffer DL (REF 740202.32; bottle of 100 mL; see ordering information)
- RNase-free-Water (REF 740378.1000 bottle of 1000 mL; see ordering information)
- Phosphate Buffered Saline (PBS)
- 50 mL conical tubes with screw cap
- Vortexer, e.g. Vortex-Genie®2 (Scientific Industries)
- Centrifuge with swing bucket rotor and 50 mL conical tube adapters (capable of 4 °C, 3000 to 4400 × *g*)

1.4 Additional buffers to be supplied for processing of EDTA / citrate blood collection tubes

- Lysis Buffer DL (REF 740202.32; bottle of 100 mL; see ordering information)
- Wash Buffer RAW (REF 740361.150; bottle of 150 mL; see ordering information)

*Please contact MACHEREY-NAGEL for technical support

1.5 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoMag® RNA Bood** kit before using this product. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure

All technical literature is available on the internet at ***www.mn-net.com***.

2 Product description

2.1 The basic principle

The **NucleoMag® RNA Blood Kit** isolates RNA from blood samples. RNA stabilizing blood collection tubes from SARSTEDT, Zymo Research and Thermo Fisher Scientific or EDTA / citrate blood collection tubes can be processed. The **NucleoMag® RNA Blood** procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by a proteinase K digestion. For the adjustment of conditions under which nucleic acids bind to the paramagnetic beads, Buffer MRB1 and the NucleoMag® B-Beads are added to the lysate. After magnetic separation and two washing steps, the paramagnetic beads are incubated with a recombinant DNase to remove co-purified DNA. Following a RNA rebinding step, residual contaminants and salts are removed with an additional washing step. Residual ethanol from previous wash step is removed by air drying. Finally, highly pure RNA is eluted with RNase-Free H_2O and the RNA can directly be used for downstream applications. The kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators

S-Monovette® RNA Exact blood collection tubes from SARSTEDT and DNA/RNA Shield™ Blood Collection Tube from Zymo Research can be processed using the same protocol. EDTA / citrate blood collection tubes and Tempus™ Blood RNA Tubes from Thermo Fisher Scientific require processing with adapted protocols (see section 5 or section 6).

2.2 Kit specifications

- The **NucleoMag® RNA Blood Kit** is designed for rapid manual and automated small-scale preparation of highly pure RNA from stabilized or EDTA / citrate blood samples. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information, section 8.2) or other magnetic separation systems (see section 2.4). Typically, 96 samples can undergo purification within 120 minutes when done manually using the NucleoMag® SEP, or in approximately 90 minutes with automated magnetic rod systems, depending on the type and configuration of the magnetic rod system.
- The purified RNA can be used directly as template for RT-PCR, or any kind of enzymatic reactions.
- Due to the recombinant DNase provided with the kit, eluted RNA is virtually DNA-free.
- For research use only.
- **NucleoMag® RNA Blood** allows easy automation on common liquid handling instruments or automated magnetic separators, for example MACHEREY-NAGEL MagnetaPure 32 Plus, IsoPure™ instruments or Thermo Fisher Scientific KingFisher®

instruments. The actual processing time depends on the configuration of the instrument and the magnetic separation system used.

- The kit provides reagents for the purification of up to 6 µg of pure RNA from suitable samples. Typical yields range between 3 µg and 4 µg RNA. Depending on the elution volume used, concentrations of 40 – 80 ng/µL can be obtained.
- **NucleoMag® RNA Blood** can be processed completely at room temperature.
- **NucleoMag® B-Beads** are highly reactive superparamagnetic beads. The binding capacity is approx. 0.4 µg / µL of RNA per 1 µL of NucleoMag® B-Bead Suspension.

2.3 Handling, preparation, and storage of starting materials

Work environment

Maintain an RNase-free work environment. Wear gloves at all times during the preparation. Change gloves frequently.

Sample storage

The protocol is optimized for the extraction of RNA from SARSTEDT S-Monovette® RNA Exact blood collection tubes, DNA/RNA Shield™ Blood Collection Tube from Zymo Research and with adaptations also from Tempus™ Blood RNA Tubes from Thermo Fisher Scientific.

Handle and store blood collection tubes according to manufacturer's instructions. Invert blood collection tubes several times before removing an aliquot for the extraction. Thaw frozen blood collection tubes from SARSTEDT, Zymo Research or Thermo Fisher Scientific immediately before RNA extraction and let them equilibrate to room temperature before removing an aliquot for the extraction. Freezing of EDTA or Citrate blood collections tubes bears the risk of RNA degradation during the thawing process.

It is highly recommended to process EDTA / citrate blood samples within a few hours after collecting them. Samples should be stored at 4 °C for no longer than 24 hours. The mRNAs contained in blood cells have different stabilities. As a result, in order to ensure that the isolated RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods before isolating RNA.

Wear gloves at all times during the preparation. Change gloves frequently.

2.4 Magnetic separation systems

For use of **NucleoMag® RNA Blood**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 8.2). The kit can also be used with other common separators. See suppliers ordering information for suitable separation plates.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481/.24)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.5 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for wash steps:

- Load 900 µL dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

2.6 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended using the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

+: acceptable, ++: good, +++: excellent, *8-channel pipetting device

2.7 Elution procedures

Purified RNA can be eluted directly with the supplied RNase-Free H_2O. Elution can be carried out in a volume of ≥ 50 µL up to 100 µL. It is essential to cover the NucleoMag® B-Beads completely with RNase-Free H_2O during the elution step. The volume of dispensed RNase-Free H_2O depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the RNase-Free H_2O.

2.8 Automation Support

MN extraction kits are designed for streamlined automation, offering compatibility with a range of leading open robotic systems. Whether you're using magnetic rod systems or liquid handlers like Hamilton, Tecan, Eppendorf, or other platforms, our kits ensure efficient and reliable extraction processes. Reach out to us for comprehensive support and tailored automation solutions, making your extraction experience seamless and hassle-free.

Questions about MACHEREY-NAGEL's scripting support or automation service?

Please contact us for personal assistance:
Phone: +49 2421 969 333
Email: support@mn-net.com

For more information, visit our website: ***www.mn-net.com/automation***

3 Storage conditions and preparation of working solutions

Attention*: Buffer MRB2 contains chaotropic salt! Wear gloves and goggles!*

- Check all components for damages after receiving the kit. If kit contents, like buffer bottles are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- The lyophilized rDNase is shipped at ambient temperature within the kit. Store lyophilized rDNAse (RNase-free) at 4 °C on arrival (stable until: see package label).
- All other kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts.
- After first use, store Liquid Proteinase K at 4 °C or -20 °C.
- All buffers are delivered ready to use.
- Before starting **NucleoMag® RNA Blood** protocol prepare the following:
- 80 % ethanol (for washing steps)
- **rDNase working solution**: Add 800 µL of RNase-free H_2O to each rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. If not used completely this working solution can be stored at -20 °C for at least 6 months. Do not freeze / thaw the rDNase working solution more than three times.
- **rDNase reaction mixture**: Add 9.2 mL Reaction Buffer for rDNase to 800 µL rDNase working solution and mix. The resulting rDNase reaction mixture will be sufficient for 32 isolations and should be used up. When performing less than 32 reactions prepare a smaller amount of the reaction mixture. For each isolation combine 276 µL of reaction buffer for rDNase with 24 µL of rDNase working solution.

	NucleoMag® RNA Blood	
REF	**1 × 96 preps 744352.1**	**4 × 96 preps 744352.4**
rDNase (lyophilized)	3 vials (size D) Add 800 µL RNase-free H_2O to each vial	12 vials (size D) Add 800 µL RNase-free H_2O to each vial

4 Safety instructions

When working with the **NucleoMag® RNA Blood** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

This manual provides detailed instructions specifically for operating the NucleoMag® RNA Blood kit. For safety guidelines related to the blood collection tubes utilized, please refer to the respective user manual. Additionally, consult the Safety Data Sheets provided for any chemicals contained within these tubes. General safety and handling instructions can be found in the corresponding user manual for the blood collection tube.

Caution: Biological samples have the potential to transmit infectious diseases. The waste generated with the **NucleoMag® RNA Blood** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to stringent lysis conditions but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to local safety regulations.

4.1 Disposal

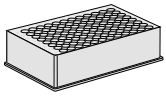
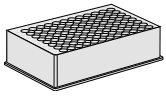
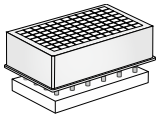
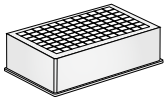
Dispose hazardous, infectious, or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

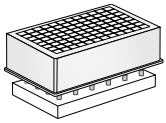
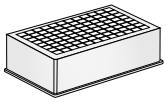
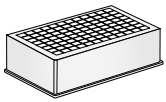
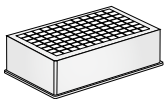
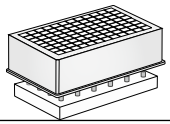
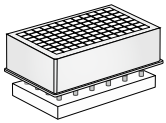
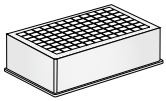
5 RNA isolation from blood collected in SARSTEDT S-Monovette® RNA Exact or Zymo DNA/RNA Shield™ blood collection tubes

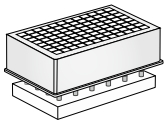
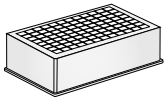
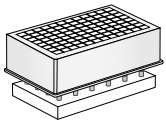
- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 17.

Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1	**Lyse** samples	**820 µL blood from SARSTEDT S-Monovette® RNA Exact or Zymo Research DNA/RNA Shield™ Blood Collection Tube** **15 µL Liquid Proteinase K** **Mix by pipetting up and down or shaking** **Incubate for 15 min at room temperature**	
2	**Bind** RNA to NucleoMag® B-Beads	**15 µL NucleoMag® B-Beads** **140 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
3	**Wash** with MRB2	Remove Square-well Block from NucleoMag® SEP **900 µL MRB2**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	

		Remove supernatant after 2 min separation	
4	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation **Dry for 5 min at RT**	
5	**Digest** DNA	Remove Square-well Block from NucleoMag® SEP **300 µL rDNase reaction mixture**	
		Mix **Incubate 15 min at RT**	
6	**Rebind**	**250 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
7	Wash with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	

		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Remove supernatant after 2 min separation	
8	**Dry** samples	**Leave Square-well Block on NucleoMag® SEP** **Air dry 10 – 15 min at RT**	
9	**Elute** RNA	Remove Square-well Block from NucleoMag® SEP **50 – 100 µL RNase-Free H2O**	
		Shake 5 – 10 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Separate 2 min and transfer RNA into elution plate / tubes	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of RNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For further automation support, please refer to chapter 2.8.

Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1 Lyse samples

Pipet 820 µL blood from a S**ARSTEDT S-Monovette® RNA Exact** or **Zymo Research DNA/RNA Shield™ Blood Collection** Tube and add 15 µL Liquid Proteinase K

Mix by pipetting up and down 6 times or by shaking

Incubate 15 min at room temperature

2 Bind RNA to NucleoMag® B-Beads

Add **15 µL resuspended NucleoMag® B-Beads and 140 µL Buffer MRB1** to the lysed sample. **Mix** by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Remove supernatant completely.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

3 Wash with MRB2

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL Buffer MRB2** to each well and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

4 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Dry beads for **5 min** at **room temperature**. Keep the Square-well Block on the NucleoMag® SEP magnetic separator for the drying step.

5 Digest DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **300 µL rDNase reaction mixture** and resuspend the beads by pipetting up and down. Incubate for **15 min** at **room temperature**. Do not separate the beads!

6 Rebind

Add **250 µL Buffer MRB1** to each sample. **Mix** by shaking for **5 min** at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

7 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

8 Dry samples

Air dry the beads for **10 – 15 min** at **room temperature**. Leave the Square-well Block on the NucleoMag® SEP magnetic separator.

9 Elute RNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **RNase-Free H_2O (50 – 100 µL)** and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times).

Incubate the suspension for **5 min** at **room temperature**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified RNA to a suitable collection plate (see ordering information, section 8.2).

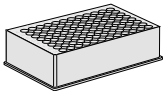
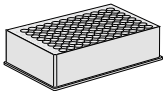
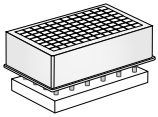
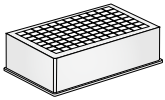
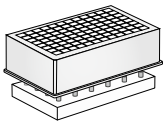
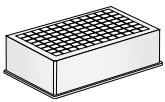
6 RNA isolation from blood collected in Tempus™ Blood RNA Tubes

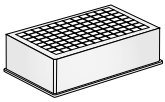
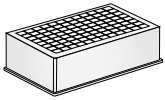
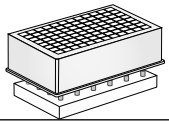
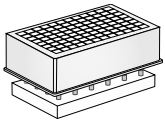
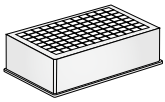
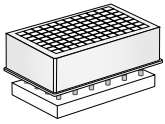
- Additional Lysis Buffer DL (REF 740202.32, see ordering information) and RNase-free-Water (e.g REF 740378.10000) are required, which are not included in the NucleoMag® RNA Blood kit
- For additional equipment and hardware requirements, refer to section 1.2 and 1.3.
- For detailed information on each step, see page 24.

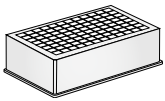
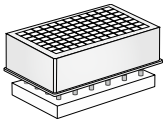
Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1	**Dilute** Sample	Poor the entire content of a Tempus™ Blood RNA Tube into a 50 mL conical tube Add 3 mL PBS to the conical tube, close the lid	
		Mix for 30 sec by vortexing at vigorous speed	
2	**Concentrate RNA**	Centrifuge for 30 min at 3000 to 4400 × g at 4 °C	
		Carefully poor the supernatant Leave the tube inverted on an absorbent paper for 1 min, blot remaining liquid from the rim *<u>Note:</u> Please handle each tube with care to avoid disrupting the crude RNA pellet settled at the bottom of the tube.*	
3	**Redissolve RNA**	Add 620 µL Buffer DL Vortex or resuspend RNA from the bottom of the tube by pipetting	

4	**Lyse** samples	Predispense 200 µL RNase free water (Not provided in the kit) 620 µL resuspended RNA pellet Mix by pipetting up and down or shaking **15 µL Liquid Proteinase K** **Mix by pipetting up and down or shaking** **Incubate for 15 min at room temperature**	
5	**Bind** RNA to NucleoMag® B-Beads	**15 µL NucleoMag® B-Beads** **140 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
6	**Wash** with MRB2	Remove Square-well Block from NucleoMag® SEP **900 µL MRB2**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
7	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	

		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation **Dry for 5 min at RT**	
8	**Digest** DNA	Remove Square-well Block from NucleoMag® SEP **300 µL rDNase reaction mixture**	
		Mix **Incubate 15 min at RT**	
9	**Rebind**	**250 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
10	Wash with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	

11	**Dry** samples	**Leave Square-well Block on NucleoMag® SEP** **Air dry 10 – 15 min at RT**	
12	**Elute** RNA	Remove Square-well Block from NucleoMag® SEP **50 – 100 µL RNase-Free H2O**	
		Shake 5 – 10 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer RNA into elution plate / tubes	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of RNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For further automation support, please refer to chapter 2.8.

Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1	**Dilute samples**
	Poor the entire content - approximately 9 mL - of a Tempus™ Blood RNA Tube into a 50 mL conical tube
	Add 3 mL PBS to the conical tube to generate a total volume of 12 mL, close the lid
	Mix for 30 sec by vortexing at vigorous speed.
2	**Concentrate RNA**
	Centrifuge for 30 min at 3000 to 4400 x *g* at 4 °C using a swing out rotor in order to concentrate the RNA at the bottom of the tube
	Carefully poor the supernatant
	Note: The RNA pellet is invisible. Poor the supernatant very careful in order not to lose the RNA pellet. Dispose waste according to your local regulations.
	Put the tube inverted on an absorbent paper and leave it for 1 min. Blot remaining liquid from the rim by dabbing it very careful on the paper
	Redissolve RNA
	Add 620 µL Buffer DL to the bottom of the tube
	Vortex or resuspend RNA from the bottom of the tube by pipetting
3	**Lyse samples**
	Pre-dispense 200 µL RNase free water in new reaction container (not provided)
	Transfer the 620 µL resuspended RNA pellet in Buffer DL to the new reaction container.
	Mix by pipetting up and down or shaking before adding the Liquid Proteinase K
	Add 15 µL Liquid Proteinase K
	Mix by pipetting up and down or shaking
	Incubate for 15 min at room temperature

4 Bind RNA to NucleoMag® B-Beads

Add **15 µL resuspended NucleoMag® B-Beads and 140 µL Buffer MRB1** to the lysed sample. **Mix** by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Remove supernatant completely.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

5 Wash with MRB2

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL Buffer MRB2** to each well and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

6 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Dry beads for **5 min** at **room temperature**. Keep the Square-well Block on the NucleoMag® SEP magnetic separator for the drying step.

7 Digest DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **300 µL rDNase reaction mixture** and resuspend the beads by pipetting up and down. Incubate for **15 min** at **room temperature**. Do not separate the beads!

8 Rebind

Add **250 µL Buffer MRB1** to each sample. **Mix** by shaking for **5 min** at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

9 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

10 Dry samples

Air dry the beads for **10 – 15 min** at **room temperature**. Leave the Square-well Block on the NucleoMag® SEP magnetic separator.

11 Elute RNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **RNase-Free H_2O (50 – 100 µL)** and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times).

Incubate the suspension for **5 min** at **room temperature**.

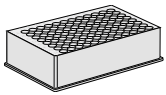
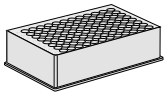
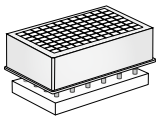
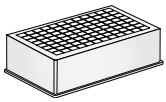
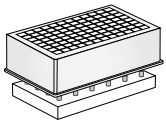
Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified RNA to a suitable collection plate (see ordering information, section 6.2).

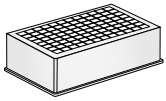
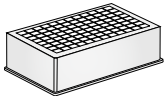
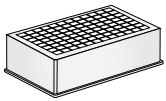
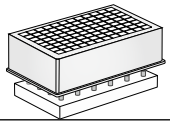
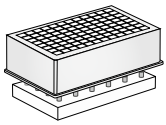
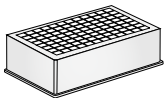
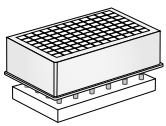
7 RNA isolation from blood collected in EDTA/ Citrate blood collection tubes

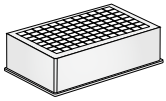
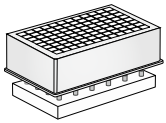
- Additional Lysis Buffer DL (REF 740202.32, see ordering information) and Wash Buffer RAW (REF 740361.150) are required, which are not included in the NucleoMag® RNA Blood kit
- For additional equipment and hardware requirements, refer to section 1.2 and 1.3.
- For detailed information on each step, see page 24.

Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1	**Lyse** samples	**260 µL blood from EDTA or Citrate blood collection tubes** **540 µl DL** **15 µL Liquid Proteinase K** **Mix by pipetting up and down or shaking** **Incubate for 15 min at room temperature**	
2	**Bind** RNA to NucleoMag® B-Beads	**15 µL NucleoMag® B-Beads** **160 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
3	**Wash** with RAW	Remove Square-well Block from NucleoMag® SEP **900 µL RAW**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	

4	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation **Dry for 5 min at RT**	
5	**Digest** DNA	Remove Square-well Block from NucleoMag® SEP **300 µL rDNase reaction mixture**	
		Mix **Incubate 15 min at RT**	
6	**Rebind**	**250 µL MRB1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
7	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **900 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	

8	**Dry** samples	**Leave Square-well Block on NucleoMag® SEP** **Air dry 10 – 15 min at RT**	
9	**Elute** RNA	Remove Square-well Block from NucleoMag® SEP **50 – 100 µL RNase-Free H_2O**	
		Shake 5 – 10 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer RNA into elution plate / tubes	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of RNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For further automation support, please refer to chapter 2.8.

Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1 Lyse samples

Pipet 260 µL blood from **a EDTA or Citrate blood collection tube** and add 540 µl Buffer DL and 15 µL Liquid Proteinase K

Mix by pipetting up and down 6 times or by shaking

Incubate 15 min at room temperature

2 Bind RNA to NucleoMag® B-Beads

Add **15 µL resuspended NucleoMag® B-Beads and 160 µL Buffer MRB1** to the lysed sample. **Mix** by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Remove supernatant completely.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

3 Wash with RAW

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL Buffer RAW** to each well and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

4 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Dry beads for **5 min** at **room temperature**. Keep the Square-well Block on the NucleoMag® SEP magnetic separator for the drying step.

5 Digest DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **300 µL rDNase reaction mixture** and resuspend the beads by pipetting up and down. Incubate for **15 min** at **room temperature**. Do not separate the beads!

6 Rebind

Add **250 µL Buffer MRB1** to each sample. **Mix** by shaking for **5 min** at room temperature or by pipetting up and down 6 times and a subsequent incubation for 5 min at room temperature.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

7 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). Incubate for **1 min**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

8 Dry samples

Air dry the beads for **10 – 15 min** at **room temperature**. Leave the Square-well Block on the NucleoMag® SEP magnetic separator.

9 Elute RNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **RNase-Free H_2O (50 – 100 µL)** and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads by repeated pipetting up and down (15 times).

Incubate the suspension for **5 min** at **room temperature**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified RNA to a suitable collection plate (see ordering information, section 8.2).

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded / no RNA obtained	*RNase contamination* • Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes or plates is recommended. Glassware should be oven-baked for at least 2 h at 250 °C before use.
Poor RNA yield	*Elution buffer volume insufficient* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step.* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation is too short or aspiration speed is too high. • Loss of RNA Pellet (**Tempus™ Blood RNA Tube**) • Make sure, that g-force and centrifugation time are sufficient and temperature is set at 4 °C. Poor supernatant very careful. Leave inverted tube only for 1 min on the absorbent paper.

Problem	Possible cause and suggestions
Low purity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example Square-well Block in combination with NucleoMag® SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* • *See above.*
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the tissue lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self adhering PE Foil (see ordering information, section 6.2) before starting the shaker.

8.2 Ordering information

Product	REF	Pack of
NucleoMag® RNA Blood	744352.1 744352.4	1 × 96 preps 4 × 96 preps
Lysis Buffer DL	740202.32	100 mL
Wash Buffer RAW	740361.150	150 mL
RNase-free Water	740378.1000	1000 mL
NucleoMag® SEP	744900	1
Square-well Blocks	740481.4 740481.24	4 24
For use of kit on KingFisher® Flex instrument: e.g., 96-well Acessory Kit B for KingFisher® (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag® RNA preps using KingFisher® Flex platform)	744951	1 set
8-place tip combs for magnetic rod systems (For use of NucleoMag® RNA Blood kit on the MagnetaPure 32 Plus or the IsoPure™ Mini instrument)	744960	50 pieces
96-Deep-Well plates for magnetic rod systems (For use of NucleoMag® RNA Blood kit on the MagnetaPure 32 Plus or the IsoPure™ Mini instrument)	744955	25 pieces

For ordering or inquiries regarding the Magnetapure 32 Plus or IsoPure™ Mini device, please contact a representative of MACHEREY-NAGEL.

Visit *www.mn-net.com* for more detailed product information.

8.3 Product use restriction/warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written

statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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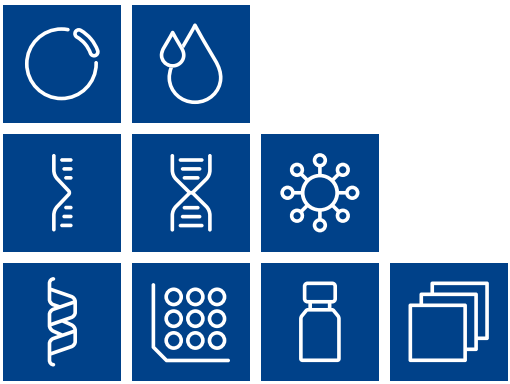
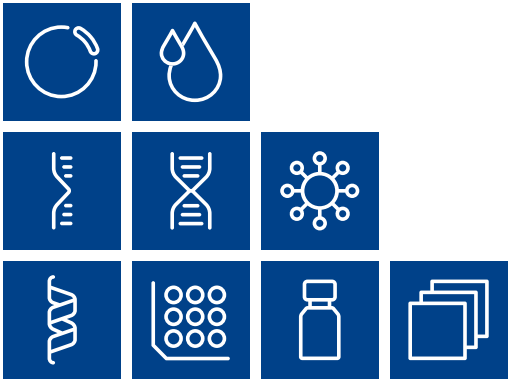


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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