

NucleoMag[®] Pathogen

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MACHEREY-NAGEL



Bioanalysis

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1 Components

1.1 Kit contents

	NucleoMag [®] Pathogen		
REF	1 × 96 preps 744210.1	4 × 96 preps 744210.4	
NucleoMag [®] B-Beads	2 × 1.5 mL	10 mL	
Lysis Buffer NPL1	30 mL	100 mL	
Binding Buffer NPB2	110 mL	3 × 110 mL	
Wash Buffer NPW3	75 mL	300 mL	
Wash Buffer NPW4	75 mL	300 mL	
Elution Buffer NPE5*	30 mL	125 mL	
Carrier RNA**	400 µg	4 × 400 µg	
Carrier RNA Buffer	500 µL	4 × 500 μL	
Proteinase K (lyophilized)**	75 mg	3 × 75 mg	
Proteinase Buffer PB	8 mL	15 mL	
User manual	1	1	

^{*} Composition of Elution Buffer NPE5: 5 mM Tris/HCl, pH 8.5

^{**} For preparation of working solutions and storage conditions see section 3.

1.2 Material to be supplied by user

Product	REF	Pack of			
Magnet for magnetic beads separation,					
e.g., NucleoMag [®] SEP NucleoMag [®] SEP Mini NucleoMag [®] SEP Maxi NucleoMag [®] SEP 24	744900 744901 744902 744903	1 1 1 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			
Lysis tubes for incubation of samples and lysis,					
e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets			
Elution plate for collecting purified nucleic acids,					
e.g., Elution Plate U-bottom (96-well 0.3 mL microtiterplate with 300 μL u-bottom wells)	740486.24	24			
For use of kit on KingFisher [®] Flex instrument:	744950	1 set			
e.g., KingFisher [®] Accessory Kit A (Deep-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag [®] Pathogen preps using KingFisher [®] Flex platform)					

Reagents:

• 80 % ethanol

1.3 About this user manual

It is strongly recommended that first time users of the **NucleoMag® Pathogen** kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used as a tool for quick referencing while performing the purification procedure.

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

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The NucleoMag[®] Pathogen kit is designed for the isolation of viral RNA and DNA and bacterial DNA from cell-free body fluids such as serum or plasma, blood, saliva, homogenized tissue sample suspensions, stool sample suspensions, and swab washes. This kit provides reagents and magnetic beads for isolation of 96 samples. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer NPL1 containing chaotropic ions supported by Proteinase K digestion. For binding of nucleic acids to the paramagnetic beads, Binding Buffer NPB2 and the NucleoMag[®] B-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers NPW3, NPW4, and 80% ethanol. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure pathogen RNA and DNA is eluted with low-salt elution buffer or water. Purified pathogen RNA and DNA can directly be used for downstream applications. It is recommended to use suitable controls for downstream applications (e.g., internal controls, extraction controls, positive / negative controls). The NucleoMag[®] Pathogen kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag® Pathogen kit is designed for rapid manual and automated small-scale preparation of viral RNA and DNA and the DNA of microorganisms from various types of clinical samples. The kit is designed for use with NucleoMag® SEP magnetic separator plate (see ordering information, section 6.2) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA and DNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions. For research use only.

NucleoMag® Pathogen kit allows easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag® SEP on an automation platform.

2.3 Magnetic separation systems

For use of **NucleoMag[®] Pathogen**, the use of the magnetic separator NucleoMag[®] SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 6.2). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag [®] SEP (MN REF 744900)	Square-well Block (MN REF 740481)
NucleoMag [®] SEP Mini (MN REF 744901)	1.5 mL or 2 mL reaction tubes (Sarstedt)
NucleoMag [®] SEP Maxi (MN REF 744902)	50 mL tubes (Falcon)
NucleoMag [®] SEP 24 (MN REF 744903)	24-Square-well Block U-bottom (MN REF 740448.4/.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. A gripper tool is required for fully automated use on liquid handling workstations. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times.

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

Beads are resuspended by the cover of the magnetic rods. Following the binding, washing and elution steps the beads are collected again with the magnetic rods.

2.4 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 crosscontamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and washing steps:

Load 600 μ L 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 s. Turn off the shaker and check the plate surface for small droplets of dyed water.

Increase speed setting, shake for an additional 30 s, and check the 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.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads into the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before dispensing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it briefly on a Vortex. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads into 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 pins, the selected separation plate, distance of the separation plate to 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 different mixing procedures. In contrast to pipetting up and down, mixing by shaker or magnetic mixing allows simultaneous washing of all samples. This reduces the time consumption and the 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

^{* 8-}channel pipetting device

2.6 Elution procedures

Purified pathogen RNA and DNA can be eluted directly with the supplied elution buffer. Elution can be carried out in a volume of \geq 50 µL. It is essential to cover the NucleoMag[®] Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer 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 elution buffer. For some separators, high elution volumes might be necessary to cover the whole pellet.

You looking for an automation solution for your NM Pathogen workflow?

Please contact: MACHEREY-NAGEL GmbH & Co. KG Tel.: +49 24 21 969-333 support@mn-net.com

3 Storage conditions and preparation of working solutions

Attention:

NPL1, NPB2 and the Carrier RNA Buffer contain chaotropic salt! Wear gloves and goggles!

All components of the $NucleoMag^{\circledast}$ Pathogen kit should be stored at room temperature (15 – 25 °C) and are stable until: see package lable.

All buffers are delivered ready-to-use.

Before starting any NucleoMag® Pathogen protocol, prepare the following:

Proteinase K: Before first use of the kit, add 3.35 mL Proteinase Buffer PB to each vial of the **Iyophilized Proteinase K**. Dissolved Proteinase K solution should be stored at -20 °C.

Carrier RNA: Before first use of the kit, add 500 µL Carrier RNA Buffer to each vial **lyophilized Carrier RNA**. Dissolve the Carrier RNA and store dissolved Carrier RNA solution in aliquots at -20 °C for up to 6 months.

<u>Note:</u> Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardy be visible.

	NucleoMag [®] Pathogen		
REF	1 × 96 preps 744210.1	4 × 96 preps 744210.4	
Proteinase K (lyophilized)	1 vial (75 mg)	3 vials (75 mg/vial)	
	Add 3.35 mL Proteinase Buffer	Add 3.35 mL Proteinase Buffer to each vial	
Carrier RNA (lyophilized)	1 vial (400 µg)	4 vials (400 µg/vial)	
	Add 500 µL Carrier RNA Buffer	Add 500 µL Carrier RNA Buffer to each vial	

4 Safety instructions

When working with the **NucleoMag® Pathogen** 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*).



Caution: Guanidine hydrochloride in buffer NPL1, and guanidinium thiocyanate in carrier RNA buffer can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoMag[®] Pathogen** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according 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 Protocol for the isolation of viral RNA and DNA and microbial DNA from blood, tissue homogenates, serum, plasma, other body fluids and washes

5.1 Preparation of sample materials

a) Blood samples / saliva

A sample volume of 100-200 μL blood is recommended. Do not use higher volumes. When processing less than 200 μL sample adjust with PBS buffer to a final volume of 200 μL .

b) Tissue samples

Homogenize tissue samples. Typically 5–10 mg sample material can be homogenized in 400 μ L PBS buffer using a bead based homogenizer. If necessary, higher amounts of sample material can be used (up to 25 mg). It should be considered that the copurified total nucleic acids may cause inhibition in the subsequent PCR assays. Centrifuge the homogenized sample and use up to 200 μ L clear supernatant for further processing. If using less than 200 μ L adjust with PBS buffer to a final volume of 200 μ L.

For isolation of viral RNA:

Tissue can also be disrupted in a buffer containing chaotropic salt (e.g., Buffer RA1, see ordering information) and beta-mercaptoethanol or TCEP reducing agent (see ordering information, section 6.2).

c) Swab samples

Incubate the swabs in PBS, sodium chloride, or cell culture medium for 30 min with agitation. Then remove the swab pressing it against the walls of the tube to squeeze out most of the liquid or use our NucleoSpin[®] Forensic Filters (single spin filters. For Lysate clearing in 96-well format use the NucleoSpin[®] Trace Filter Plate, see ordering information, section 6.2).

d) Feces

Mix 1 volume of feces (e.g., 500μ L) with an equal volume of PBS buffer. Mix vigorously by vortexing for 1 min. Allow the particles to settle down or centrifuge with low speed (e.g., at $500 \times g$). Proceed with the cleared supernatant. For difficult to lyse bacteria mechanical disruption or treatment using suitable beads may be required (e.g., Bead Tubes Type A, see ordering information section 6.2)

e) Clean up of TRIzol® purified samples

After phase separation by centrifugation proceed with the aqueous phase (the colorless upper phase; Approximately 400 μ L). For further processing start with step 2 of the purification protocol by mixing 400 μ L of the aqueous phase with 600 μ L Buffer NPB2 and 20 μ L NucleoMag[®] B-Beads.

In the processing of $\ensuremath{\text{NucleoZOL}}$, the aqueous phase can be used in the same way as described above.

5.2 Protocol at a glance

For hardware requirements refer to section 2.3.

For detailed information on each step see page 15.

Before starting the preparation:

Check if Proteinase K and Carrier RNA were prepared according to section 3.

1	Lyse sample	200 μL (homogenized) sample	
		20 µL Proteinase K	
		4 µL Carrier RNA	
		180 µL NPL1	
		Mix	
		RT, 15 min	
		or 56 °C, 15 min	
	Disalaria		~
2	Bind nucleic acids to	600 μL NPB2	
	NucleoMag [®] B-Beads	20 µL B-Beads	
		Mix by shaking for 5–10 min at RT	
		(Optional: Mix by pipetting up and down)	\Leftrightarrow
		Remove supernatant after 2 min separation	
3	Wash with	Remove Square-well	
	NPW3	Block from NucleoMag [®] SEP	
		600 µL NPW3	
		Resuspend: Shake 1 min at RT	\leftrightarrow
		Remove supernatant after 2 min separation	

4	Wash with NPW4	Remove Square-well Block from NucleoMag [®] SEP 600 μL NPW4	
		Resuspend: Shake 1 min at RT	\leftrightarrow
		Remove supernatant after 2 min separation	
5	Wash with 80 % Ethanol	Remove Square-well Block from NucleoMag [®] SEP	
		600 μL 80 % Ethanol	
		Resuspend: Shake 1 min at RT	↔
		Remove supernatant after 2 min separation	
6	Drying step	Air dry for 10 min at room-temperature	
7	Elute RNA and DNA	Remove Square-well Block from NucleoMag [®] SEP	
		50-100 μL NPE5	
		Shake 5 min at RT	
		(Optional: Mix by pipetting up and down)	
		Separate 2 min and transfer RNA and DNA into elution plate / tubes	

5.3 Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag[®] SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see ordering information, section 6.2). Alternatively, isolation of pathogen RNA and DNA 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.

1 Lyse sample

Pre-dispense 20 μL Proteinase K and 200 μL of sample to a suitable reaction tube. Add 180 μL Lysis Buffer NPL1 to the reaction tube.

Optional: Add 4 µL of the Carrier RNA stock solution to the reaction tube.

Mix well by repeated pipetting up and down and incubate at room temperature for 15 min with shaking. Alternatively, lysis step can be performed in Tube Strips (see ordering information, section 6.2).

Following the lysis incubation, spin down to collect any sample from the lysis tube lids and transfer each lysate to the wells of a Square-well Block.

Lysis incubation can be performed at 56 °C to increase the lysis efficiency e.g. for isolation of bacterial DNA from difficult to lyse bacteria.

Optionally, lysis can be supported by a pretreatment of the sample with suitable beads for mechanical disruption of difficult to lyse bacteria.

2 Bind nucleic acids to magnetic beads

Add 20 μL resuspended NucleoMag® B-Beads and 600 μL Binding Buffer NPB2 to the lysed sample.

Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**.

Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

NucleoMag[®] B-Beads and Buffer NPB2 can be premixed.

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

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

Do not disturb the attracted beads while aspirating the supernatant.

3 Wash with NPW3

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

Add **600 \muL Buffer NPW3** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

4 Wash with NPW4

Remove the Square-well Block from the NucleoMag[®] SEP magnetic separator. Add **600 \muL Buffer NPW4** and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

5 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag[®] SEP magnetic separator. Add 600 μ L 80 % ethanol and resuspend the beads by shaking until the beads are resuspended completely (1 – 3 min). Alternatively, resuspend beads completely by repeated pipetting up and down.

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

6 Air dry magnetic beads

Air dry the magnetic bead pellet for 10 min at room temperature.

7 Elute RNA and DNA

Remove the Square-well Block from the NucleoMag[®] SEP magnetic separator. Add desired volume of NPE5 (50–100 μ L) to each well of the Square-well Block and resuspend the beads by shaking 5 min at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for 5 min at 56 °C.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag[®] SEP magnetic separator. Wait at least **2 min** until all beads have been attracted to the magnets. Transfer the supernatant containing the purified nucleic acids to either microtubes or Tube Strips (see ordering information, section 6.2).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions		
	Incomplete sample lysis		
	• Sample mixed with Lysis Buffer and Proteinase K was not thoroughly homogenized and mixed with Lysis buffer, Proteinase K. The mixture has to be shaken continuously. Alternatively, prolong incubation time with Proteinase K.		
	Insufficient elution buffer volume		
	• Bead pellet must be covered completely with elution buffer and needs to be fully resuspended.		
	Insufficient performance of elution buffer during elution step		
Poor yield / low sensitivity	• Remove all buffer completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps.		
	Aspiration of attracted bead pellet		
	• Do not disturb the attracted beads while aspirating the supernatant. This requires special caution when removing the lysate from the beads as the lysate is usually too opaque to allow visual control of the pellet.		
	Aspiration and loss of beads		
	• Time for magnetic separation too short or aspiration speed too high.		
	Insufficient washing procedure		
Low purity/low sensitivity	 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.		

Problem	Possible cause and suggestions		
	Carry-over of ethanol from wash buffers		
Poor performance of DNA/RNA	 Be sure to remove all of the 80 % ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications. 		
in downstream applications	Ethanol evaporation from wash buffers		
αρρισαιοπο	 Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs. 		
	Time for magnetic separation too short		
Carry-over of	 Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. 		
beads	Aspiration speed too high (elution step)		
	High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.		

6.2 Ordering	information
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Product	REF	Pack of
NucleoMag [®] Pathogen	744210.1 744210.4	1 × 96 preps 4 × 96 preps
NucleoMag [®] SEP	744900	1
NucleoMag [®] SEP Mini	744901	1
NucleoMag [®] SEPMaxi	744902	1
NucleoMag [®] SEP 24	744903	1
Square-well Blocks	740481 740481.24	4 24
Self-adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each, and 12 Cap Strips)	740477 740477.24	4 sets 24 sets
NucleoSpin [®] Forensic Filters (Semipermeable mini spin filter; individually blistered)	740988.50 740988.250	50 250
NucleoSpin [®] Forensic Filters (Bulk) (Semipermeable mini spin filter; bulk packed)	740988.50B 740988.250B 740988.1000B	50 250 1000
NucleoSpin [®] Trace Filter Plate (96-well filter plate to e.g., separate swabs from the lysate)	740677	20
NucleoSpin [®] Bead Tubes Type A	740786.50	50
96-well Accessory Kit A for KingFisher Square-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag [®] Pathogen preps using KingFisher [®] Flex platform	744950	1 set
Buffer RA1 (60 mL)	740961	60 mL
Reducing agent TCEP	740395.107	107 mg

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

6.3 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.

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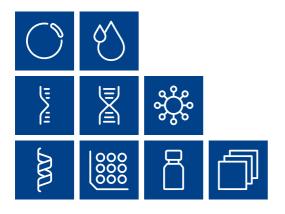
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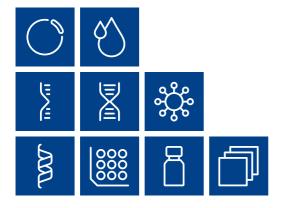
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Plasmid DNA Clean up RNA DNA Viral RNA and DNA Protein High throughput Accessories Auxiliary tools



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