Bioanalysis

MACHEREY-NAGEL

User manual

Genomic DNA from tissue

■ NucleoMag® Tissue

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1 Components

1.1 Kit contents

	NucleoMag® Tissue		
REF	**1 × 96 preps 744300.1**	**4 × 96 preps 744300.4**	**24 × 96 preps 744300.24**
NucleoMag® B-Beads	2 × 1.5 mL	12 mL	70 mL
Lysis Buffer T1	50 mL	100 mL	1000 mL
Binding Buffer MB2	45 mL	180 mL	2 × 500 mL
Wash Buffer MB3	75 mL	300 mL	2 × 900 mL
Wash Buffer MB4	75 mL	300 mL	2 × 900 mL
Wash Buffer MB5	125 mL	500 mL	3 × 1000 mL
Elution Buffer MB6	30 mL	125 mL	2 × 500 mL
Proteinase K (lyophilized)*	75 mg	4 × 75 mg	24 × 75 mg
Proteinase Buffer PB	8 mL	15 mL	3 × 35 mL
User manual	1	1	1

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

1.2 Equipment and consumables to be supplied by user

Product	REF	Pack of
Magnetic separation system e.g., NucleoMag® SEP (see section 2.3)	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
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) e.g., Elution Plate Flat-bottom (96-well 0.3 mL microtiterplate with 370 µL flat-bottom wells)	740486.24	24
For use of kit on KingFisher® Flex instrument: KingFisher® Accessory Kit A (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 × 96 NucleoMag® Tissue preps using KingFisher® Flex platform)	744950	1 set
For use of kit on KingFisher® Duo / Duo Prime instrument: KingFisher® Duo Accessory Kit (KingFisher® Deep-well Blocks, KingFisher® Duo 12 Tip Combs, KingFisher® Duo Elution Strips for 8 × 12 NucleoMag® Tissue preps using KingFisher® Duo / Duo Prime platform)	744952	1 set

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at *www.mn-net.com*.

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

2 Product description

2.1 The basic principle

The **NucleoMag® Tissue** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Tissue samples, cells, or bacteria are lysed with SDS / Proteinase K solution (Buffer T1). For the adjustment of the binding conditions under which nucleic acids bind to the paramagnetic beads, Buffer MB2 and the NucleoMag® B-Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed twice to remove contaminants and salts using Wash Buffer MB3 and Wash Buffer MB4. There is no need for a drying step as ethanol from previous wash steps is removed by a final incubation of the beads in Buffer MB5. Finally, highly purified DNA is eluted with low salt elution buffer (Buffer MB6) and can directly be used for downstream applications. The **NucleoMag® Tissue** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag® Tissue is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from tissue samples, cells or bacteria using the NucleoMag® SEP (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.

NucleoMag® Tissue allows easy automation on common liquid handling instruments. 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 the automation platform.

The kit provides reagents for the purification of up to 20 µg of pure genomic DNA from suitable samples (up to 20 mg tissue, up to 1×10^6 cells or up to 1 mL of an overnight culture of bacteria) with an A_{260}/A_{280} ratio ≥ 1.6 – 1.9 and typical concentration of 20 – 50 ng / µL. Depending on the elution volume used, concentrations of 10 – 150 ng / µL can be obtained.

Following lysis of samples with Proteinase K, **NucleoMag® Tissue** can be processed completely at room temperature, however, elution at 55 °C will increase the yield by about 15 – 20 %.

For research use only.

2.3 Magnetic separation systems

For use of **NucleoMag® Tissue**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). 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/.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.

2.4 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 cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

- Load 1000 µL (for checking the settings for the binding step) or 600 µL (for checking the settings for the washing steps) 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 the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, 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 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

2.6 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer MB6. Elution can be carried out in a volume of ≥ 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, higher elution volumes might be necessary to cover the whole pellet.

Elution is possible at room temperature. Yield can be increased by 15 – 20 % if elution is performed at 55 °C.

For more information on processing, please visit the following websites.
www.mn-net.com/magneticbeadprocessing

3 Storage conditions and preparation of working solutions

Attention: *Buffers MB2, MB3, and MB4 contain chaotropic salt! Wear gloves and goggles!*

Storage conditions:

- All components of the **NucleoMag® Tissue** kit should be stored at room temperature (15 – 25 °C) and are stable until: see package label.
- All buffers are delivered ready to use.

Before starting any **NucleoMag® Tissue** protocol, prepare the following:

- **Proteinase K:** Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for at least 6 months.

	NucleoMag® Tissue		
REF	**1 × 96 preps 744300.1**	**4 × 96 preps 744300.4**	**24 × 96 preps 744300.24**
Proteinase K (lyophilized)	75 mg Add 2.6 mL Proteinase Buffer	4 × 75 mg Add 2.6 mL Proteinase Buffer to each vial	24 × 75 mg Add 2.6 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoMag® Tissue** 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 *www.mn-net.com/msds*).

Caution: Sodium perchlorate in buffer MB2, MB3 and MB4 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® Tissue** 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.

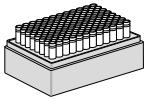
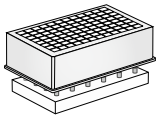
5 Protocol for the isolation of genomic DNA from tissue

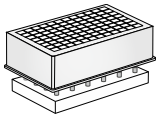
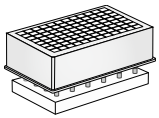
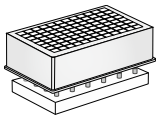
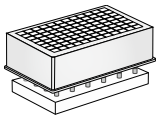
Protocol at a glance

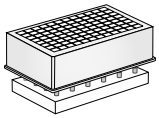
- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 14.

Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.

1	**Lyse samples** (up to 20 mg tissue, up to 1 × 10^6 cells or bacteria pellet from up to1 mL overnight culture)	**25 µL Proteinase K** **200 µL T1** **Mix** **56 °C, 1 – 3 h or overnight**	
2	**Clear lysates** by centrifugation, transfer 225 µL of cleared lysate to a Square-well Block for further processing	**5,600 x g, 5 min** **225 µL cleared lysate**	 
3	**Bind** DNA to NucleoMag® B-Beads	**24 µL NucleoMag® B-Beads** **360 µL MB2**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	

4	**Wash** with MB3	Remove Square-well Block from NucleoMag® SEP **600 µL MB3**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
5	**Wash** with MB4	Remove Square-well Block from NucleoMag® SEP **600 µL MB4**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
6 !	**Wash** with MB5	<u>Leave</u> Square-well Block on NucleoMag® SEP **900 µL MB5** **Incubate for 45 – 60 s** *<u>Note:</u> Do not resuspend the beads in Buffer MB5!*	
		Remove supernatant	

7	Elute DNA	Remove Square-well Block from NucleoMag® SEP **50 – 200 µL MB6** *(Optional: Elute at 55 °C)*	
		Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Separate 2 min and transfer DNA 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 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.

Before starting the preparation:

- Check if Proteinase K was prepared according to section 3.

1 Lyse samples

Calculate the amount of lysis stock required: for each sample **25 µL of Proteinase K solution +200 µL Buffer T1** are required. Prepare lysis stock solution accordingly and vortex.

Never prepare the lysis stock solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in Buffer T1 without substrate.

Transfer 225 µL of the resulting stock solution to each lysis tube containing up to 20 mg of tissue sample (e.g., mouse tail section), or up to 1×10^6 cultured cells or up to 1 mL of an overnight culture of bacteria. Close the individual tubes. Mix by vigorous shaking for 10 – 15 s. Spin briefly (15 s; 1,500 x *g*) to collect any sample at the bottom of the tube.

The sample must be submerged in the solution.

Incubate the tubes containing the samples at 56 °C until complete lysis is obtained (at least 1 – 3 h or overnight). For cultured cells, incubation can be carried out at 70 °C for 10 – 15 min. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes are securely closed.

If RNA-free DNA is crucial for downstream applications, an RNase digest may be performed: add 20 µL RNase A (20 mg / mL) solution (not included, see ordering information) and incubate for additional 5 min at room temperature.

2 Clear lysates

Centrifuge the samples for **5 min** at a full speed (**5,600 – 6,000 x *g***). Remove cap strips.

Transfer **225 µL of the cleared lysate** (equilibrated to room temperature) to a Square-well Block. Do not moisten the rims of the well.

Note: See recommendations for suitable plates or tubes and compatible magnetic separators section 1.2.

3 Bind DNA to NucleoMag® B-Beads

Add **24 µL of NucleoMag® B-Beads** and **360 µL Buffer MB2** to each well of the Square-well Block. 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.

Note: NucleoMag® B-Beads and Buffer MB2 can be premixed before use. Premix just before use, storage of premixed beads and buffers is not recommended. Mix 24 µL NucleoMag® B-Beads with 360 µL Buffer B2 per sample. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are necessary. Use 384 µL of the suspension per well. 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 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.

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.

4 Wash with MB3

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

Add **600 µL Buffer MB3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

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 magnet. Remove and discard supernatant by pipetting.

5 Wash with MB4

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

Add **600 µL Buffer MB4** to each well and resuspend the beads by shaking until the beads are resuspended completely (5 min). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

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 magnet. Remove and discard supernatant by pipetting.

6 Wash with MB5

Leave the Square-well Block on the NucleoMag® SEP magnetic separator.

! *Note: Supernatant is colorless, magnetic bead pellet is clearly visible.*

Gently add **900 µL Buffer MB5** to each well and incubate for **45 – 60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MB5. This step is to remove traces of ethanol and eliminates a drying step!

7 Elution

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

Add desired volume of **Buffer MB6 (50 – 200 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 – 10 min** at **56 °C**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5 – 10 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 the beads have been attracted to the magnets. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

Note: Yield can be increased by 15 – 20 % by using pre-warmed elution buffer (55 °C) or by incubating the bead / elution buffer suspension at 55 °C for 10 min.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA 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 efficiency of following wash steps and elution step. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Partial elution in Wash Buffer MB5 already* • Keep the beads on the magnet while dispensing Wash Buffer MB5. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the DNA already. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Incubation after dispensing beads to lysate* • Mix immediately after dispensing NucleoMag® B-Beads / Buffer MB2 to the lysate.
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.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol wash solutions* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* • See above

Problem	Possible cause and suggestions
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) before starting the shaker.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® Tissue	744300.1 744300.4 744300.24	1 × 96 preps 4 × 96 preps 24 × 96 preps
Buffer T1	740940.25 740940.100 740940.1000	50 mL 100 mL 1000 mL
RNase A	740505.50	50 mg
NucleoMag® SEP	744900	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
Elution Plate U-bottom	740486.24	24
Elution Plate Flat-bottom	740673	20

Product	REF	Pack of
96-well Accessory Kit A for KingFisher® (set consists of Square-well Blocks, Deep-well Tip Combs, Elution Plates; for 4 × 96 NucleoMag® Tissue preps using KingFisher® Flex platform)	744950	1 set

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.

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-NAGELS 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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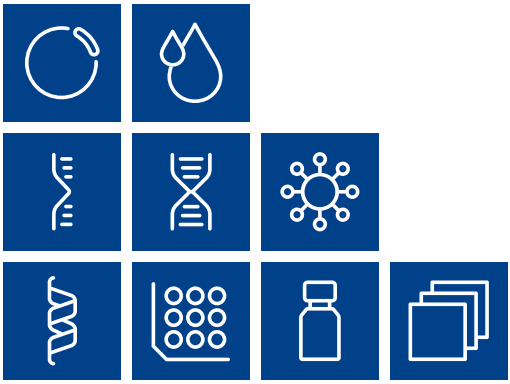
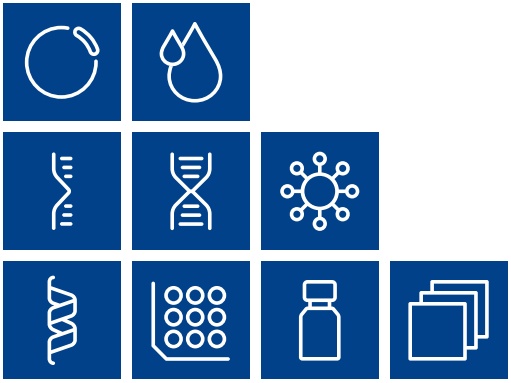


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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