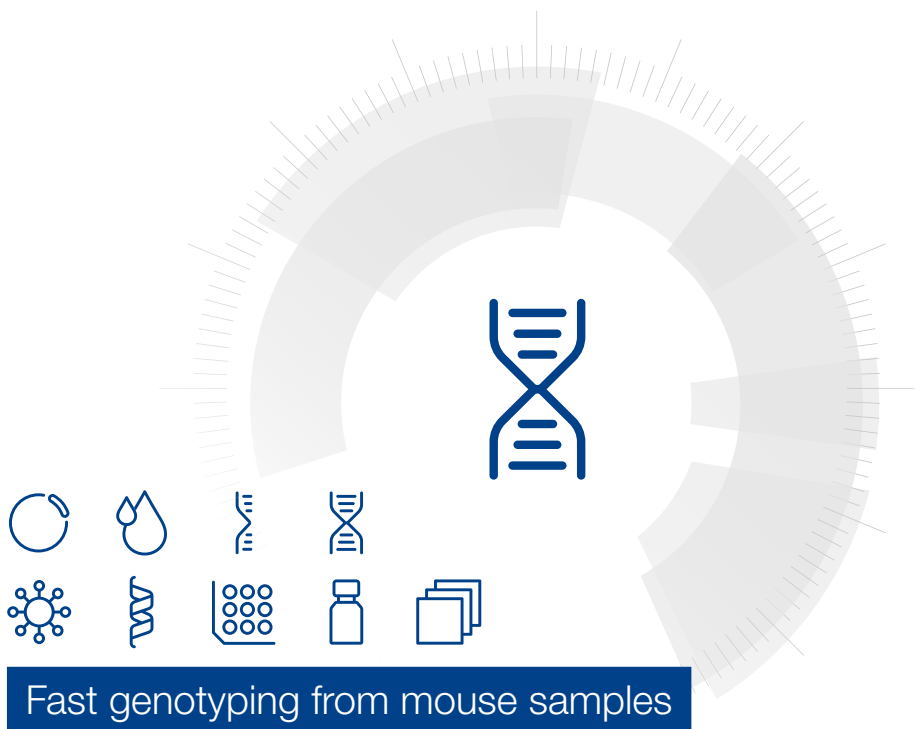


MACHEREY-NAGEL

User manual



■ NucleoType Mouse PCR

November 2023 / Rev. 03

MACHEREY-NAGEL

www.mn-net.com



Contact MN

Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
E-mail: info@mn-net.com

Technical Support Bioanalysis

Tel.: +49 24 21 969-333
E-mail: support@mn-net.com

USA

MACHEREY-NAGEL Inc.
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA
Toll-free: 888 321 6224 (MACH)
E-mail: sales-us@mn-net.com

France

MACHEREY-NAGEL SAS
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €
Siret 379 859 531 00020 · RCS Strasbourg B379859531 · N° intracommunautaire FR04 379 859 531

Switzerland

MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

Table of contents

1 Components	4
1.1 Kit contents	4
1.2 Reagents, consumables, and equipment to be supplied by user	4
1.3 About this user manual	5
2 Product description	5
2.1 The basic principle	5
2.2 Kit specifications	6
2.3 Handling, preparation, and storage of starting materials	6
2.4 Lysis and disruption of sample material	6
3 Storage conditions and preparations of working solutions	7
4 Safety instructions	7
4.1 Disposal	7
5 Protocols	8
5.1 Mouse typing with tail clip, ear punch, or hair	8
5.2 Mouse typing with blood	9
5.3 Reaction setup for single-plex 10 µL PCR	10
5.4 Reaction setup for single-plex 5 µL PCR	11
5.5 PCR cycling parameters	12
6 Analysis of PCR products	14
7 Appendix	15
7.1 Troubleshooting	15
7.2 Ordering Information	16
7.3 Product use restriction / warranty	17

1 Components

1.1 Kit contents

NucleoType Mouse PCR			
REF	25 preps 743200.25	100 preps 743200.100	500 preps 743200.500
Lysis Buffer M	12 mL	12 mL	60 mL
Liquid Proteinase K	50 µL	50 µL	250 µL
NucleoType HotStart PCR Master Mix (2x) (containing polymerase, dNTPs, buffer, enhancer, stabilizer)	125 µL	500 µL	2 × 1250 µL
User manual	1	1	1

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- Primer for mouse specific target of interest
- Water (PCR grade; for primer dilution and reaction fill-up)

Consumables

- Disposable pipette tips
- 1.5 mL or 2.0 mL microcentrifuge tube (for lysis)
- PCR tubes

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes (optional)
- Shaker (for lysis, recommended)
- Thermal heating block recommended (for Proteinase K inactivation), or heated water bath
- Personal protection equipment (lab coat, gloves, goggles)
- PCR machine

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the NucleoType Mouse PCR kit before using this product.

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

2 Product description

2.1 The basic principle

Many mouse genotyping methods are based on PCR amplification of genes of interest from tail biopsies or ear punches. The NucleoType Mouse PCR kit is designed for rapid mouse typing experiments and covers these standard sample materials including even blood and animal friendly hair samples.

Hair samples can be easily collected causing minimal discomfort to the animal and do not require anesthesia. Thus, the NucleoType Mouse PCR kit enables animal friendly and time saving mouse genotyping.

A simple sample preparation within one tube is combined with a fast PCR. Mouse samples (tail clipping, ear punch, blood, or fur hair) are subjected to a quick lysis in a small volume and short time (< 5 min). For optimal results an aliquot from the lysate is used as template for PCR typing. The enzyme mix enables cycling times of less than one hour. Analysis of the amplicons can be performed by gel electrophoresis or Bioanalyzer®. The reactions contain a dye and are ready to load directly on the agarose gel. There is no need to add a dye in order to assist application of the amplicon onto the gel. The dye of the NucleoType Mouse PCR kit is compatible with Bioanalyzer® (Agilent DNA 1000 Kit). Using a fast cycler, simple sample preparation and the subsequent PCR can be performed in less than one hour. In addition the NucleoType Mouse PCR kit offers an alternative direct PCR protocol allowing PCR directly from unpurified and undiluted samples.

2.2 Kit specifications

Kit specifications at a glance

Parameter	NucleoType Mouse PCR kit
Technology	Simple sample preparation suitable for hot start PCR and direct PCR
Format	20 – 100 µL lysate; 10 µL PCR (optional 5 – 50 µL)
Sample type	Mouse tail clipping, mouse ear punch, mouse blood (fresh or frozen EDTA, citrate, heparin, or untreated), mouse hair
Sample amount	1 mm diameter ear punch, 1 mm outer tail clipping, or small tuft of hair (approximately 3 – 30 hairs), or 1 µL mouse blood
Typical yield	Strong bands in agarose gel electrophoresis, approximately 100 ng to 600 ng amplicon per 10 µL PCR (primer and target dependent)
Amplicon size	Up to 1000 bp
Preparation time	Simple sample preparation in less than 5 min PCR cycling: 30 – 90 min (cycler and target size dependent)
Analysis	Gel electrophoresis: Approx. 30 min (40 samples); Bioanalyzer®: Approx. 40 min (12 samples)
Use	For research use only

2.3 Handling, preparation, and storage of starting materials

The kit is designed to perform genotyping on the following sample materials: Mouse tail clipping, mouse ear punch, mouse hair, and mouse blood.

Fresh or frozen material can be used.

Respect your local animal welfare regulations when choosing, harvesting and handling mouse samples!

2.4 Lysis and disruption of sample material

In order to obtain reliable mouse typing data, it is important to obtain sufficient amount of DNA in a form suitable to serve as template for subsequent PCR amplification. The NucleoType Mouse PCR kit contains an optimized Lysis Buffer M for sufficient DNA release within a short 2 minute lysis step. For optimal PCR results we recommend to use an aliquot of this lysate as template for the PCR typing. Optional, the NucleoType Mouse PCR kit offers an alternative direct PCR protocol allowing PCR directly from unpurified and undiluted samples (see section 5.1 and 5.2).

3 Storage conditions and preparations of working solutions

The NucleoType Mouse PCR kit should be stored upon arrival at +4 °C or -20 °C. The kit is stable for at least 12 month when stored at this temperature. The kit can be shipped at ambient temperature for up to up to 3 month. Short time exposure (up to 14 days) at temperatures up to 37 ° is tolerable.

After first time usage, store all kit components at +4 °C or -20 °C.

The Lysis Buffer M, Liquid Proteinase K and NucleoType HotStart PCR Master Mix (2x) are ready to use.

Store NucleoType HotStart PCR Master Mix in the dark, e.g. within the product box in a freezer (- 20 °C; recommended) or fridge (+ 4 °C). Avoid prolonged exposure of the mix to light. Setting up PCR at average laboratory illumination is tolerable. Do not expose the mix to direct sunlight.

Prepare a primer mix containing primer for your target of interest. Recommended concentration per primer: See section 5.3 and 5.4.

4 Safety instructions

Use the product according to the user manual.

The product does not contain components requiring GHS hazard or precaution phrases

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 Protocols

5.1 Mouse typing with tail clip, ear punch, or hair

Lysis protocol (recommended)

1 Prepare final Lysis Buffer

Add **20 µL Lysis Buffer M** into a lysis tube (1.5 or 2.0 mL; not provided).

Add **0.5 µL Liquid Proteinase K** into the Lysis Buffer M.

Note: If several samples are processed at a time, a premix of Lysis Buffer M and Liquid Proteinase K can be prepared, e.g., 200 µL Lysis Buffer M with 5 µL Liquid Proteinase K. Such a premix is stable for at least 1 h at room temperature (18–25 °C).

2 Incubate and lyse sample

Place a **mouse typing sample** into the lysis tube

Mouse tail clip: Approximately 1 mm from the outer end of the tail.

Mouse ear punch: 1 mm in diameter ear punch. Use e.g. a biopsy punch 1 mm such as PF49101 Disposal Biopsy Punches with Plunger-System (pmf Medical).

Note: Ear punches with a smaller diameter (e.g., 0.3 mm) are hard to handle and placing of such small samples into the Lysis Buffer M may be imprecisely. Therefore such small samples are not recommended. Ear punches with a diameter larger than approximately 1.5 mm should not be used as they might overload the reaction.

Mouse hair: Place one tuft of hair (approximately 3–30 fur hairs) into the lysis tube. Make sure to place the hairs at the bottom of the tube, preferably roots down! Short centrifugation of the closed lysis tube may help to spin down the hairs. A slant tweezer with a 1 × 3 mm flat area to grab the fur hairs is recommended.

Incubate 2 min shaking at ambient temperature (18–25 °C; DNA release step).

Note: If continuous shaking is not possible, a motionless incubation can be performed, however an initial shaking is required.

Note: Incubation times of 1–5 min are possible and show similar results.

3 Deactivate Proteinase K

Incubate 2–3 min at 98 °C (Proteinase K inactivation step).

4 Store sample or prepare PCR reaction mixture

Centrifuge the lysate briefly in order to sediment debris.

Note: Typically, a centrifugation step is not necessary. Residual tissue might still be visible but this does not impair the subsequent reaction.

The lysate can be stored for at least 2 weeks at room temperature (18–25 °C), 2 month at 4 °C or 2 years at - 20 °C. Repeated freeze-thaw cycles are unproblematic.

Use 1 µL liquid of this lysate as template for the subsequent PCR.

Note: Take care not to transfer residual tissue pieces.

For the direct PCR protocol place the sample (1 mm diameter ear punch, 1 mm outer tail clipping, or small tuft of hair; approximately 3–30 hairs) directly into the PCR reaction (50 µL of volume). Make sure that the sample is covered by the PCR Mix.

5.2 Mouse typing with blood

Lysis protocol (recommended)

1 Prepare final Lysis Buffer

Add **100 µL Lysis Buffer M** into a lysis tube (1.5 or 2.0 mL; not provided).

Add **0.5 µL Liquid Proteinase K** into the Lysis Buffer M.

Note: If several samples are processed at a time, a premix of Lysis Buffer M and Proteinase K can be prepared, e.g., 1000 µL Lysis Buffer M with 5 µL Liquid Proteinase K. Such a premix is stable for at least 1 h at room temperature (18–25 °C).

2 Incubate and lyse sample

Add **1 µL mouse blood (fresh or frozen EDTA, citrate, heparin or untreated) as typing sample** into the lysis tube.

Incubate 2 min shaking at ambient temperature (18–25 °C; DNA release step).

Note: If continuous shaking is not possible, a motionless incubation can be performed, however an initial mixing is required

Note: Incubation times of 1–5 min are possible and show similar results.

3 Deactivate Proteinase K

Incubate 2–3 min at 98 °C (Proteinase K inactivation step).

4 Store sample or prepare PCR reaction mixture

Centrifuge the lysate briefly in order to sediment debris.

Note: Typically, a centrifugation step is not necessary. Residual blood might still be visible but this does not impair subsequent reaction.

The lysate can be stored for at least 2 weeks at room temperature (18–25 °C), 2 month at 4 °C or 2 years at - 20 °C. Repeated freeze-thaw cycles are unproblematic.

Use 1 µL liquid of this lysate as template for the subsequent PCR.

For the direct PCR protocol place the sample (1 µL of a 1:20 diluted blood sample) directly into the PCR reaction (50 µL of volume).

5.3 Reaction setup for single-plex 10 µL PCR

The 10 µL reaction is the recommended standard reaction volume for the NucleoType Mouse PCR kit. Due to the hot start technology of this product, the reaction set up can be performed at room temperature (18–25 °C).

Per reaction combine the following:

5 µL NucleoType HotStart PCR Master Mix (2x)

2 µL forward primer (stock concentration 1 µM or 1 pmol/µL)

2 µL reverse primer (stock concentration 1 µM or 1 pmol/µL)

1 µL of mouse sample lysate

→10 µL final PCR volume

A final concentration of 0.2 µM per primer is recommended.

The addition of silicone oil is not necessary and will impair removal of the liquid after the reaction. Therefore, the addition of silicone oil is not recommended.

Note: Depending on the PCR tubes used, the initial 10 µL set up volume might shrink to approximately 8 µL due to evaporation during cycling. This has been taken into account and does not impair the reaction.

*Note: If desired, the final **PCR volume can be scaled up** by increasing all components proportionally.*

If **duplex PCR** is set up, adjust primer concentration to 0.2 µM as final concentration for each primer. Use e.g.,

1 µL forward primer (stock concentration 2 µM) for target one

1 µL reverse primer (stock concentration 2 µM) for target one

1 µL forward primer (stock concentration 2 µM) for target two

1 µL reverse primer (stock concentration 2 µM) for target two

5.4 Reaction setup for single-plex 5 µL PCR

Due to the hot start technology of this product, the reaction set up can be performed at room temperature (18–25 °C). Per reaction combine the following:

2.5 µL NucleoType HotStart PCR Master Mix (2x)

0.1 µL forward primer (stock concentration 10 µM or 10 pmol/µL)

0.1 µL reverse primer (stock concentration 10 µM or 10 pmol/µL)

1.0 µL of mouse sample lysate

1.3 µL water

→5 µL final PCR volume

Note: The addition of silicone oil is not necessary and will impair removal of the liquid after the reaction. Therefore, the addition of silicone oil is not recommended.

Note: Depending on the PCR tubes used, the initial 5 µL set up volume might shrink to approximately 4 µL due to evaporation during cycling. This has been taken into account and does not impair the reaction.

PCR - Reaction setup overview

Component	5 µL PCR rxn	10 µL PCR rxn (recommended)	50 µL PCR rxn (direct PCR) tissue	50 µL PCR rxn (direct PCR) blood
NucleoType HotStart PCR Master Mix (2x)	2.5 µL	5 µL	25 µL	25 µL
Forward primer	0.1 µL (conc. 10 µM or 10 pmol/µL)	2 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)
Reverse primer	0.1 µL (conc. 10 µM or 10 pmol/µL)	2 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)	10 µL (conc. 1 µM or 1 pmol/µL)
Sample (see section 5.1 and 5.2)	1 µL lysate	1 µL lysate	mouse tail clip ear punch, fur	1 µL of 1:20 diluted mouse blood
H ₂ O	1.3 µL	-	5 µL	4 µL

5.5 PCR cycling parameters

Cycling conditions are depending on primer and PCR machine set up. For several primer pairs with primer T_m ranging from 40 °C to 75 °C the following PCR programs have been used successfully.

For amplicons from 50–1000 bp an initial extension time of approximately 60 seconds is recommended.

Note: For initial testing annealing time of 20 s and extension time of 60 s is recommended. For amplification of fragments smaller 1000 bp and / or for amplifications with a PCR machine with slow ramp rates (e.g. 2 °C/s) annealing / and extension time may successively be reduced (e.g. to 15 s annealing and 15 s extension). PCR machines with fast ramp rates (e.g. 5 °C/s) may not be reduced as much as for slow ramping machines.

PCR program 1 (three step program for typical endpoint PCR machines)

Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C	15 s	40 cycles
	40–75 °C*	20 s	
	72 °C	60 s	
Extension	72 °C	1 min	1 cycle
Cooling	4 °C		
Total time		ca. 70–100 min (total run time is annealing temperature and machine dependent)	

PCR program 2 (Two step program for typical end point PCR machines)

Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C	15 s	40 cycles
	60–72° C*	60 s	
Extension	72 °C	1 min	1 cycle
Cooling	4 °C		
Total time		ca. 66 min (machine dependent)	

*: Optimal annealing temperature is primer dependent and has to be determined empirically. A good starting point for testing is 50 °C. Optimally, a good annealing temperature for primer of your choice is determined with a temperature gradient cyclor.

PCR program 3 (e.g., LightCycler® 1.5, in glas capillary)

Initial Denaturation	95 °C	2 min	1 cycle
Amplification	95 °C	15 s	40 cycles
	40–75 °C*	15 s	
	72 °C	30 s	
Extension	72 °C	1 min	1 cycle
Cooling	20 °C		
Total time		ca. 30–60 min (annealing temperature dependent)	

Note: The LightCycler® is used herein solely as a fast cycling instrument, but not for quantitative PCR!

Note: It is recommended to target sequences not exceeding 500 bp in glass capillaries.

6 Analysis of PCR products

The PCR products (amplicons) can be directly analyzed by one of the following methods.

Gel electrophoresis: Apply the total PCR reaction onto a agarose gel (e.g. 1 % agarose) for analysis.

There is no need to add loading dye for gel electrophoresis, because the PCR mix already contains a dye and suitable density.

There is no need to perform a proteinase digestion step prior to analysis of the amplicons.

Dye migration in

- 1 % agarose gel: Approximately as 600 bp fragment
- 2 % agarose gel: Approximately as 350 bp fragment

Bioanalyzer® (Agilent): Use 1 µL with e.g., the Agilent DNA 1000 Kit.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Reduction of initial 10 µL PCR set up volume during PCR cycling	<ul style="list-style-type: none"> Depending on tightness of PCR tubes used, the initial 10 µL PCR set up volume might shrink to approximately 8 µL. This is acceptable and does not impair typing performance. If volume reduction is even more pronounced, use a tighter reaction tube.
No amplicon detected	<i>Unfavorable primer selection.</i>
	<ul style="list-style-type: none"> Make sure that the primer are selected well and are able to amplify the desired target from 1 – 10 ng of purified genomic DNA. Test different primer annealing temperatures.
	<i>Unfavorable storage conditions</i>
	<ul style="list-style-type: none"> Store NucleoType HotStart PCR Master Mix in the dark, e.g. within the product box in a freezer (- 20 °C; recommended) or fridge (+ 4 °C). Avoid prolonged exposure of the mix to light. Setting up PCR at average laboratory illumination is tolerable. Do not expose the mix to direct sunlight.
	<i>Too much extract in PCR.</i>
	<ul style="list-style-type: none"> Make sure to use at most 1 µL lysate as template per reaction.
	<i>Liquid Proteinase K was skipped or heat incubation step was skipped.</i>
	<ul style="list-style-type: none"> Make sure to add Liquid Proteinase K to the Lysis Buffer M and make sure to incubate for at least 2 min at 98 °C in order to inactivate the Proteinase K.
	<i>PCR cycling conditions not optimal.</i>
	<ul style="list-style-type: none"> Decrease annealing temperature. Test different primer annealing temperatures. Increase extension time. Increase number of cycles up to 40.
Too little amplicon yield	<ul style="list-style-type: none"> Try to adjust annealing temperature and extension time
Amplicon does not have the correct size	<ul style="list-style-type: none"> Make sure that the primers are selected well and are able to amplify the desired fragment from 1 – 10 ng of purified genomic template DNA.

Problem	Possible cause and suggestions
Two amplicons of different sizes are expected, but only one band is observed by agarose gel electrophoresis	<ul style="list-style-type: none">• Make sure that the analysis method has enough resolving power to discriminate the two different sizes of DNA fragments. Use Bioanalyzer® instead of gel electrophoresis or increase electrophoresis time or gel concentration.• Make sure that both primer pairs have a similar amplification efficiency. If this is not the case, titrate down the primer pair yielding an amplicon (use a smaller concentration for this primer pair).

7.2 Ordering Information

Product	REF	Pack of
NucleoType Mouse PCR	743200.25	25 reactions x 10 µL
NucleoType Mouse PCR	743200.100	100 reactions x 10 µL
NucleoType Mouse PCR	743200.500	500 reactions x 10 µL

7.3 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.

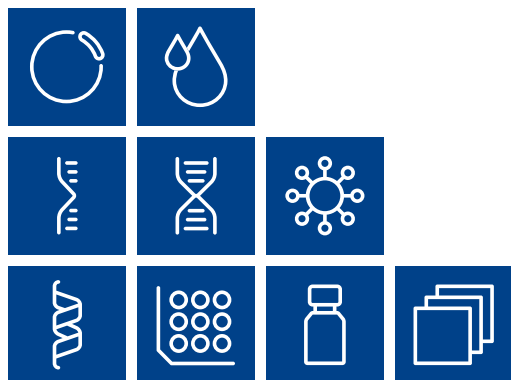
This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No 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 customer 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.

Last updated: 08/2022, Rev. 04

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



Plasmid DNA

Clean up

RNA

DNA

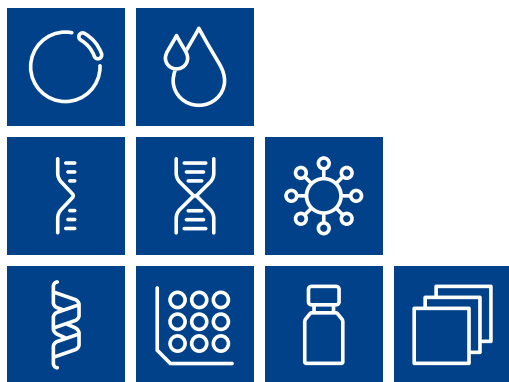
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



www.mn-net.com

MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany

DE +49 24 21 969-0 info@mn-net.com

CH +41 62 388 55 00 sales-ch@mn-net.com

FR +33 388 68 22 68 sales-fr@mn-net.com

US +1 888 321 62 24 sales-us@mn-net.com