



Fast genotyping from blood samples

User manual

NucleoType Blood PCR

May 2020 / Rev. 02

Contact MN

Germany and international

MACHEREY-NAGEL GmbH & Co. KG

Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

Tel.: +49 24 21 969-0

Toll-free: 0800 26 16 000 (Germany only)

Fax: +49 24 21 969-199

E-mail: info@mn-net.com

Technical Support Bioanalysis

Tel.: +49 24 21 969-270

E-mail: tech-bio@mn-net.com

USA

MACHEREY-NAGEL Inc.

2850 Emrick Blvd. · Bethlehem, PA 18020 · USA

Tel.: +1 484 821 0984

Toll-free: 888 321 6224 (MACH)

Fax: +1 484 821 1272

E-mail: sales-us@mn-net.com

France

MACHEREY-NAGEL SARL à associé unique

1, rue Gutenberg · 67722 Hoerdt · France

Tel.: +33 388 68 22 68

Fax: +33 388 51 76 88

E-mail: sales-fr@mn-net.com

Switzerland

MACHEREY-NAGEL AG

Hirsackerstr. 7 · 4702 Oensingen · Switzerland

Tel.: +41 62 388 55 00

Fax: +41 62 388 55 05

E-mail: sales-ch@mn-net.com

www.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	4
2	Product description	5
2.1	The basic principle	5
2.2	Kit specifications	5
2.3	Handling, preparation, and storage of starting materials	5
2.4	Lysis and disruption of sample material	6
3	Storage conditions and preparations of working solutions	6
4	Safety instructions	6
5	Protocols	7
5.1	Blood typing without Inhibitor Removal Pearls with many blood samples (e.g. human, cat, sheep, guinea pig, cow)	7
5.2	Blood typing with Inhibitor Removal Pearls for challenging blood samples (e.g. mouse, rat, chicken, rabbit)	8
5.3	Blood typing with blood storage cards	9
5.4	Reaction setup for 10 μ L PCR (single-plex or duplex)	9
5.5	PCR cycling parameters	10
6	Analysis of PCR products	11
7	Appendix	12
7.1	Troubleshooting	12
7.2	Ordering information	13
7.3	Product use restriction / warranty	13

1 Components

1.1 Kit contents

NucleoType Blood PCR Kit			
REF	25 preps 743201.25	100 preps 743201.100	500 preps 743201.500
Blood Transfer Tool (BTT)	25 pieces	100 pieces	500 pieces
Inhibitor Removal Pearls (IRP)	25 pieces	100 pieces	500 pieces
NucleoType HotStart PCR Master mix (2x) (containing polymerase, dNTPs, buffer, enhancer, stabilizer)	125 µL	500 µL	2 x 1250 µL
User Manual	1	1	1

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

Reagents

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

Consumables

- Disposable pipette tips
- PCR tubes

Equipment

- Manual pipettes
- Vortexer (to mix blood samples after addition of Inhibitor Removal Pearl)
- Personal protection equipment (lab coat, gloves, goggles)
- PCR machine
- Gel electrophoretic equipment or Bioanalyzer® for analysis of generated amplicons

1.3 About this user manual

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

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

Please contact technical service regarding information about any changes to the current user manual compared with previous revisions.

2 Product description

2.1 The basic principle

Many blood genotyping methods are based on DNA purification out of whole blood, followed by PCR amplification of genes of interest. However, DNA purification from whole blood is a time consuming and elaborate process.

The NucleoType Blood PCR kit is designed for rapid blood typing experiments using whole blood (treated with EDTA, citrate, or heparin as anticoagulant) and blood dried on blood cards as sample material, without the need to purify DNA from blood.

From liquid blood sample material a standardized blood aliquot is directly transferred into the PCR via the Blood Transfer Tool (BTT), which is supplied in the kit. This procedure enables easy and fast genotyping for many different kind of blood samples (e.g., human, cat, lamb, cattle). Some blood types which are even more challenging (e.g., rabbit, mouse, rat, chicken) can also easily be processed by the addition of an Inhibitor Removal Pearl (provided in the kit) to the blood sample before withdrawal of a blood aliquot for PCR.

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoType Blood PCR kit
Technology	Direct PCR: Transfer of blood aliquot with Blood Transfer Tool (BTT) into PCR
Format	10 µL PCR (optional up to 50 µL)
Sample type	Whole blood from e. g., human, mouse, rat, cat, chicken, rabbit, guinea pig, sheep, or cow treated with EDTA, citrate, or heparin as anticoagulant. Punches from blood storage cards like NucleoCard® (MN) and FTA cards (Whatman).
Preparation time	Sample preparation: 0–1 min; PCR cycling: 30–90 min (cyclers and target size dependent)
Amplicon size	Up to 1000 bp
Analysis	Gel electrophoresis: Approx. 30 min (40 samples); Bioanalyzer®: Approx. 40 min (12 samples)

2.3 Handling, preparation, and storage of starting materials

The kit is designed to perform genotyping on the following sample materials: Whole blood treated with EDTA, citrate, or heparin as anticoagulant or without anticoagulant. Fresh and frozen blood can be used.

Punches from blood storage cards like NucleoCard® (MN) and FTA cards (Whatman) can be used.

Respect you local regulations when choosing, harvesting and handling your blood samples.

2.4 Lysis and disruption of sample material

No special step for lysis or disruption of blood samples is required: A blood aliquot is directly applied to the PCR with the Blood Transfer Tool (BTT) (provided in the kit).

3 Storage conditions and preparations of working solutions

The NucleoType Blood 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 (18 °C–25 °C) for up to 3 month. Short time exposure (up to 14 days) at temperatures up to 37 °C is tolerable.

After first time usage, store all kit components at +4 °C or -20 °C. The NucleoType HotStart PCR Master Mix (2x) is 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. For recommended primer concentrations see section 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

5 Protocols

5.1 Blood typing without Inhibitor Removal Pearls with many blood samples (e.g. human, cat, sheep, guinea pig, cow)

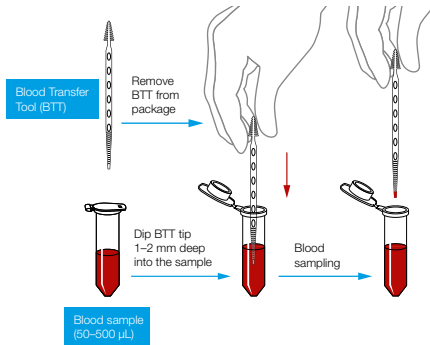
Prepare sample

Mix the blood sample so that all constituents are evenly distributed within the blood.

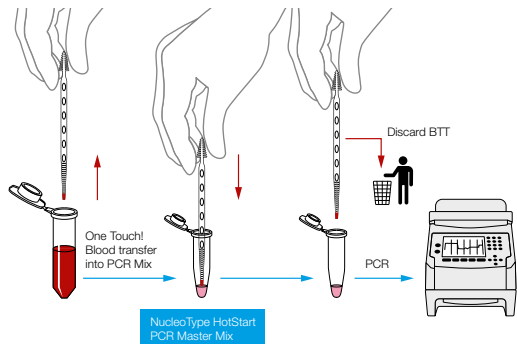
Blood transfer

For every blood sample:

Remove one Blood Transfer Tool (BTT) from its package, briefly touch the blood surface with the pinpoint end of the tool (stick it approximately 1–2 mm deep into the blood).



Insert the pinpoint tip adhering the blood aliquot briefly (one-touch, approx. 1 second, do not stir in) into the prepared PCR mix and discard the Blood Transfer Tool (BTT) properly (respecting your local regulations for blood handling).



5.2 Blood typing with Inhibitor Removal Pearls for challenging blood samples (e.g. mouse, rat, chicken, rabbit)

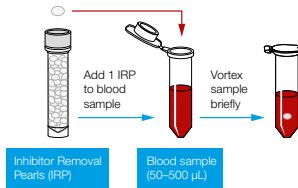
Prepare sample

Mix the blood sample so that all constituents are evenly distributed within the blood.

To a **50 µL–500 µL blood** aliquot add **one Inhibitor Removal Pearl (IRP)**.

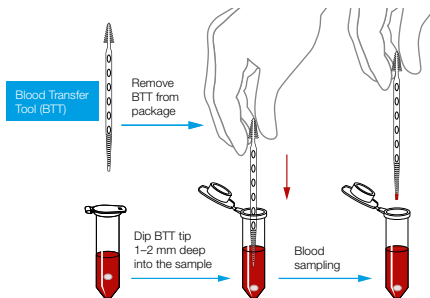
Mix briefly (e.g., by vortexing) and incubate for at least **15 seconds**.

Note: The IRP treated blood sample can be used directly or stored at -20 °C for several month or at +4 °C for some weeks. Immediately before use, vortex the blood sample!

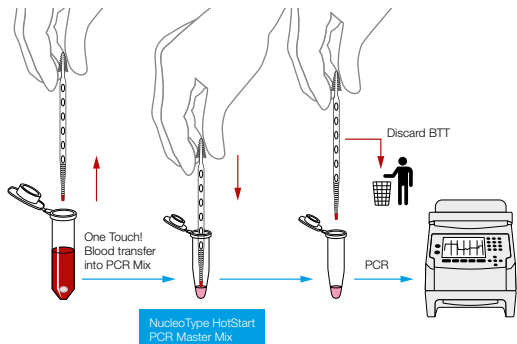


Blood transfer

For every blood sample, remove one Blood Transfer Tool (BTT) from its package, briefly touch the blood surface with the pinpoint end of the tool (stick it approximately **1–2 mm** deep into the blood).



Insert the pinpoint tip adhering the blood aliquot briefly (one-touch, approx. 1 second, do not stir in) into the prepared PCR mix and discard the Blood Transfer Tool (BTT) properly (respecting your local regulations for blood handling).



5.3 Blood typing with blood storage cards

Prepare sample

Take a punch of approximately **0.3–1 mm** disc from the blood spot of the blood storage card.

Sample transfer

Add the punch directly into the prepared PCR mix. A PCR volume of **20 µL–50 µL** is recommended.

5.4 Reaction setup for 10 µL PCR (single-plex or duplex)

The 10 µL reaction is the recommended, standard reaction volume for the NucleoType Blood 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).

5 µL primer mix (each primer with a concentration of 0.4 µM within the 5 µL primer mix, resulting in a final concentration of 0.2 µM in the PCR per primer).

→ 10 µL final PCR volume, ready to receive the blood aliquot from the Blood Transfer Tool (BTT).

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

5.5 PCR cycling parameters

Cycling conditions are depending on primer and PCR machine set up. For several primer pairs with 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		approx. 70–100 min (total run time is annealing temperature and machine dependent)	

PCR program 2 (two step program for typical endpoint 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		approx. 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 cyler.

** : The optimal annealing / extension time is primer dependent. Only primer with melting temperature above 60 °C are recommended for this program.

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		approx. 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.

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.

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

*: 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 cyler.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Reduction of initial 10 μ L PCR set up volume during PCR cycling	<ul style="list-style-type: none"> Depending on the PCR tube size, 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 smaller reaction tube.
	<p><i>Unfavorable primer selection.</i></p> <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. <p><i>Unfavorable storage conditions</i></p> <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.
No amplicon detected	<p><i>Too much blood in PCR.</i></p> <ul style="list-style-type: none"> Make sure to transfer the blood sample with the Blood Transfer Tool (BTT), which ensures the dispensing of a small, suitable blood aliquot. Pipet tips are not recommended for blood transfer. <p><i>Too much blood card in PCR</i></p> <ul style="list-style-type: none"> Make sure to use a 0.3–1 mm punch of a blood card in a 20–50 μL PCR. Make sure that the punch actually enters the PCR solution before starting the program. <p><i>Challenging blood sample</i></p> <ul style="list-style-type: none"> Some blood types, e.g. mouse, rat and chicken are especially challenging samples. Follow the procedure using Inhibitor Removal Pearls! <p><i>Too short annealing and/or extension time</i></p> <ul style="list-style-type: none"> Increase annealing and/or extension time. PCR machines with fast ramp rates (e.g. 5°C/s) typically require somewhat longer annealing / extension times than machines with slow ramp rates (e.g. 1°C/s). <p><i>PCR cycling conditions not optimal.</i></p> <ul style="list-style-type: none"> Decrease annealing temperature. Test different primer annealing temperatures. Increase extension time. Increase number of cycles up to 40

Problem	Possible cause and suggestions
Too little amplicon yield	<ul style="list-style-type: none"> • Try to adjust annealing temperature and extension time or follow the procedure using the Inhibitor Removal Pearls
Amplicon does not have the correct size	<ul style="list-style-type: none"> • Make sure that the primer are selected well and are able to amplify the desired targeted from 1–10 ng of purified genomic DNA.
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 Blood PCR	743201.25	25 reactions x 10 µL
NucleoType Blood PCR	743201.100	100 reactions x 10 µL
NucleoType Blood PCR	743201.500	500 reactions x 10 µL

7.3 Product use restriction / warranty

NucleoType Blood PCR kit components were developed, designed and sold for research purposes only. They are suitable for in vitro uses only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE

WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent 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 the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Please contact:
MACHEREY-NAGEL Germany
Tel.: +49 (0) 24 21 969-270
e-mail: TECH-BIO@mn-net.com

Trademarks / disclaimer:

Bioanalyzer® is a registered trademark of Agilent, Inc.
Eppendorf® is a registered trademark of Eppendorf AG.
LightCycler® is a trademark of ROCHE DIAGNOSTICS GMBH

All used names and denotations can be brands, trademarks or registered labels of their respective owner-also if they are not special denotation. To mention products and brands is only a kind of information, i.e. it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment. Regarding these products or services we can not grant any guarantees regarding selection, efficiency or operation.



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 · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany

DE / International:

Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:

Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:

Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:

Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

