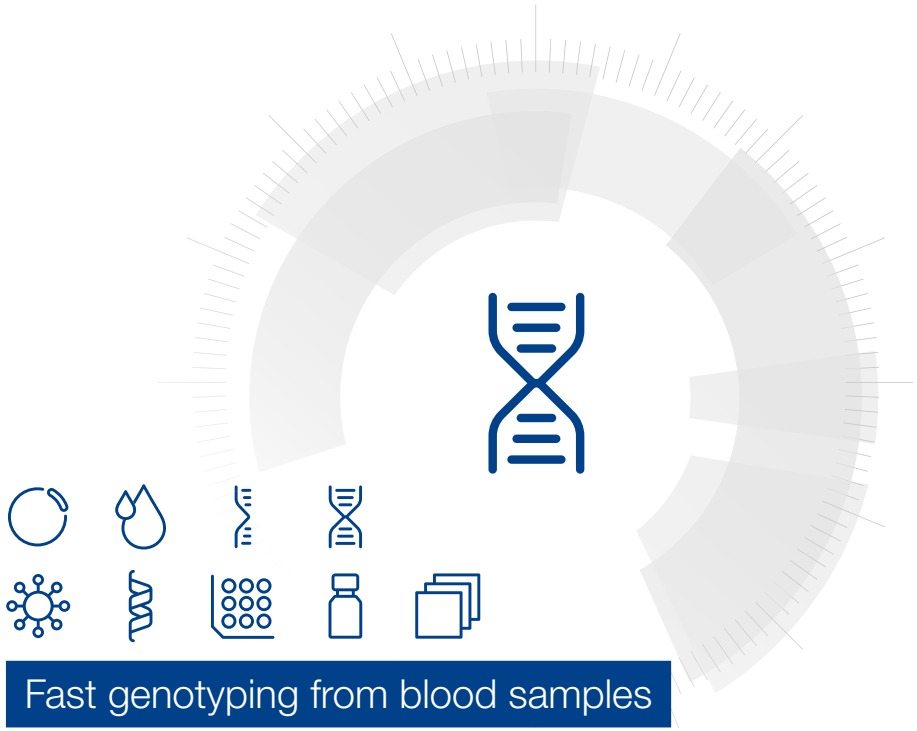
Bioanalysis

MACHEREY-NAGEL

User manual



Fast genotyping from blood samples

■ NucleoType Blood PCR

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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 × 1250 µL
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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 fist 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 (cycler and target size dependent)
Amplicon size	Up to 1000 bp
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: 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

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

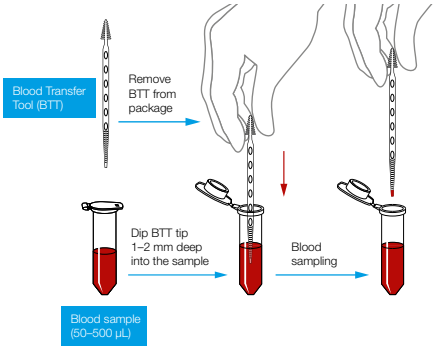
Prepare sample

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

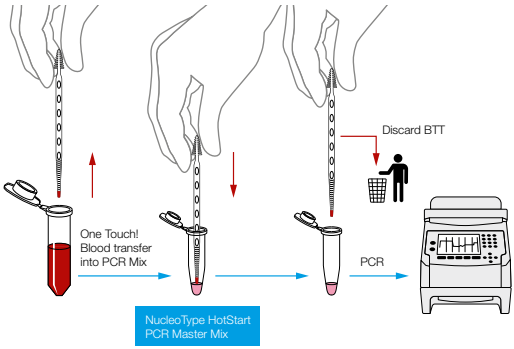
Blood transfer

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)

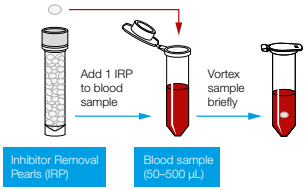
Prepare sample

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)**.

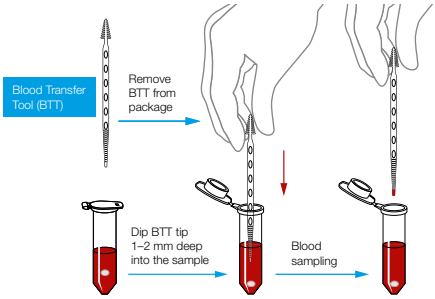
Mix briefly (e.g., by vortexing) and incubate for at least **15 seconds.**

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!

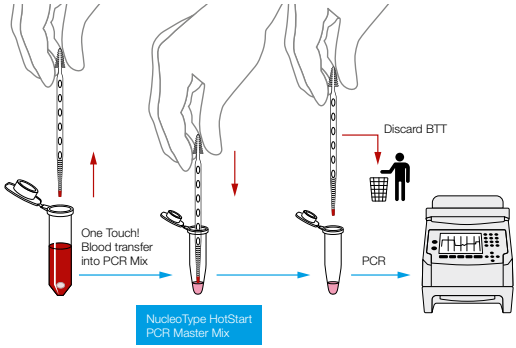


Blood transfer

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 40 – 75 °C* 72 °C	15 s 20 s 60 s	40 cycles
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 end point PCR machines)			
Initial denaturation	95 °C	2 min	1 cycle
Amplification	95 °C 60 – 72° C**	15 s 60 s	40 cycles
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 cycler.

**: 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 40 – 75 °C* 72 °C	15 s 15 s 30 s	40 cycles
Extension	72 °C	1 min	1 cycle
Cooling	20 °C		
Total time		approx. 30 – 60 min (anneling 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 cycler.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Reduction of initial 10 µL PCR set up volume during PCR cycling	• 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.
No amplicon detected	*Unfavorable primer selection.* • 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. *Unfavorable storage conditions* • 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. *Too much blood in PCR.* • 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. *Too much blood card in PCR* • 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. *Challenging blood sample* • Some blood types, e.g. mouse, rat and chicken are especially challenging samples. Follow the procedure using Inhibitor Removal Pearls! *Too short annealing and / or extension time* • 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). *PCR cycling conditions not optimal.* • 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	• Try to adjust annealing temperature and extension time or follow the procedure using the Inhibitor Removal Pearls
Amplicon does not have the correct size	• Make sure that the primer are selected well and are able to amplify the desired targed 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.	• 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

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

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

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

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact

your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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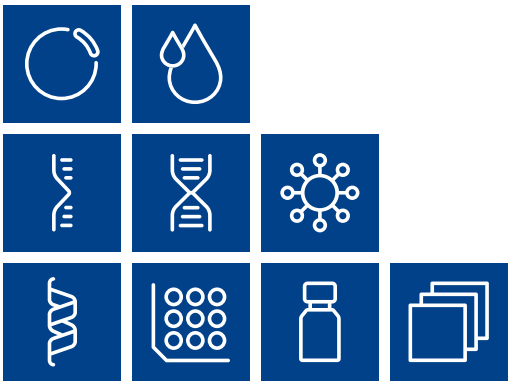
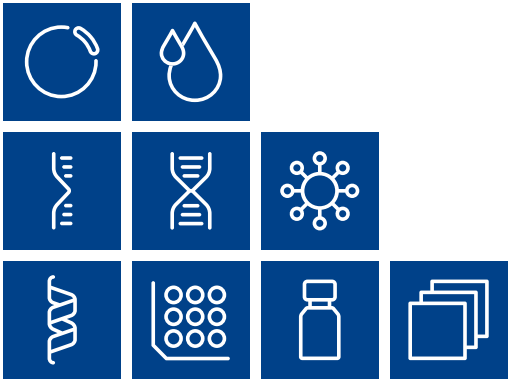


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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