NucleoType Blood PCR

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

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

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

Material supplied by the 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 electrophoresis chamber or Bioanalyzer®

Kit specifications

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 EDT, 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)
Analysis	Gel electrophoresis: Approx. 30 min (40 samples); Bioanalyzer®: Approx. 40 min (12 samples)
Dye migration	1 % agarose gel: Approximately as 600 bp fragment 2 % agarose gel: Approximately as 350 bp fragment

The basic principle

Many blood genotyping methods are based on PCR amplification of genes of interest from DNA purified out of whole blood. 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 fresh or frozen whole blood (treated with EDTA, citrate, or heparin as anticoagulant) and blood dried on blood cards like NucleoCard® (MN) and FTA™ cards (Whatman™) 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 an blood aliquot for PCR.

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 (provided in the kit).

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

Safety instructions

Use the product according to the user manual.

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

PCR cycling parameters

Cycling conditions are depending on primer and PCR machine set up. For several primer pairs with primer Tm 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 approx. 60 s is recommended.

Note: For initial testing annealing / extension time of 20 / 60 s is recommended. For amplification of fragments smaller 1000 bp and/or using a slow ramp rate e.g. 2 °C/s PCR machine annealing / extension time may successively be reduced (e.g. to 15 s annealing, 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)	

* 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.

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 / 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)	

* 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.

Note: The LightCycler® is used herein solely as a fast cycling instrument, but nor for quantitative PCR!

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

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.

Product use restriction / warranty

NucleoType Blood PCR kit components are intended, 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. 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.

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.

For more detailed product use restriction / warranty please have a look at: ***www.mn-net.com***.

Blood typing for common blood sample types (e.g., human, cat, sheep, guinea pig, cow)

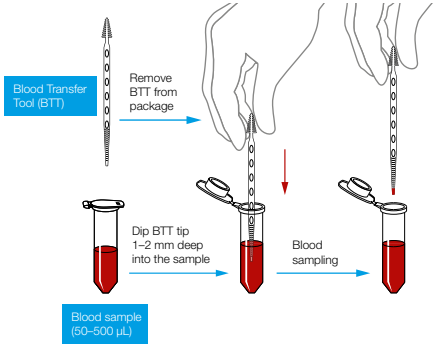
Prepare sample

Mix the blood sample to evenly distribute constituents within the blood.

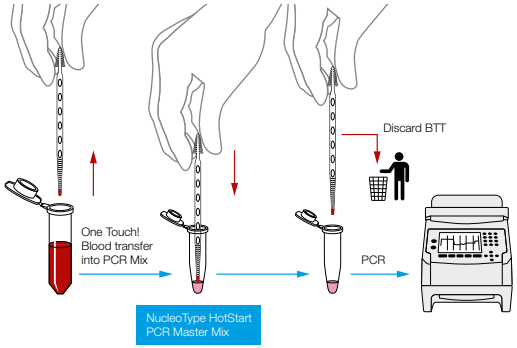
Blood transfer

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. 1s, do not stir in) into the prepared PCR mix and discard the Blood Transfer Tool (BTT) properly (respecting your local regulations for blood handling).



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.

Prepare sample

Add the punch directly into the prepared PCR mix. PCR of 20 µL–50 µL final volume are recommended.

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

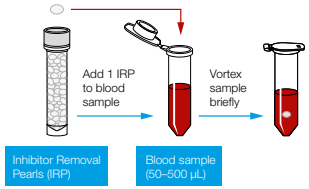
Prepare sample

Mix the blood sample to evenly distribute constituents within the blood.

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

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

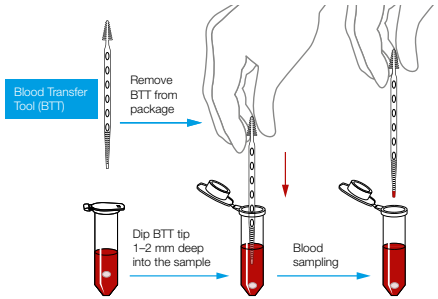
Note: The thus treated blood sample can be used directly or stored at -20 °C for several month or at +4 °C for some weeks. Immediately before use, vortex the blood sample!



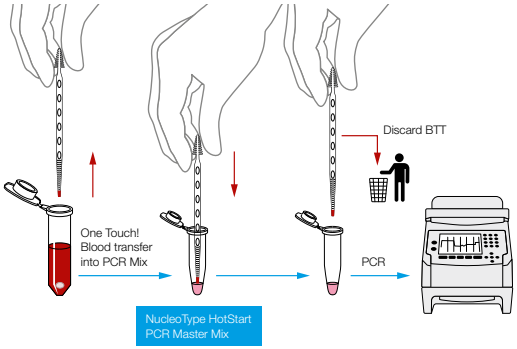
Blood transfer

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 s, do not stir in) into the prepared PCR mix and discard the Blood Transfer Tool (BTT) properly (respecting your local regulations for blood handling).



Reaction setup for single-plex 10 µL PCR

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.

Troubleshooting

Reduction of initial 10 µL PCR set up volume during PCR cycling

- 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

- *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.
- *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! (IRP)
- *PCR cycling conditions not optimal*
  Decrease annealing temperature. Test different primer annealing temperatures. Increase extension time. Increase number of cycles up to 40
- *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).

Too little amplicon yield

- Try to adjust annealing temperature and extension time.

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 e.g., 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).

Trademarks / disclaimer:

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