Bioanalysis

MACHEREY-NAGEL

User manual

Ready-to-use Hot Start Master Mix for PCR

- NucleoType® HotStart PCR Master Mix

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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

www.mn-net.com

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1 Components

1.1 Kit contents

NucleoType® HotStart PCR Master Mix (2x): 2 × 1.25 mL REF 743215

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

Reagents

- Target-specific primers
- 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, e.g. in combination with NucleoType® DirectLyse Reagent REF 743220)
- Transfer tools (for colony PCR, e.g. pin, needle, toothpick)
- PCR tubes

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes (optional)
- Shaker (recommended for lysis with NucleoType® DirectLyse)
- Thermal heating block recommended (for Proteinase K inactivation according to NucleoType® DirectLyse), 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® HotStart PCR Master Mix kit before using this product. All technical literature is available online at ***www.mn-net.com***.

2 Product description

2.1 The basic principle – intended use

The NucleoType® HotStart PCR Master Mix is a PCR mix containing Polymerase, dNTPs, buffer, enhancer, stabilizer, and a colorant. The mix utilizes superior developments in buffer chemistry and polymerase technology to improve PCR yield, specificity, and speed. The enzyme utilizes advanced hot-start technology for excellent sensitivity.

The enzyme and buffer system allow for high end PCR performance on complex templates such as mammalian genomic DNA. Due to high efficiency and specificity, the enzyme is perfectly suited to multiplex PCR assays. Due to the presence of a colorant, PCR reactions can be directly loaded onto agarose gels without additional loading buffer.

The NucleoType® HotStart PCR Master Mix is a sturdy mix for every day PCR applications including genotyping, muliplex PCR, screening, library construction, colony PCR, and direct PCR from blood, tissue and food samples.

The NucleoType® HotStart PCR Master Mix is recommended to be used either for colony PCR reactions (please see chapter 5.1 for detailed information) or in combination with one of the following NucleoType® products (please see ordering information, chapter 7.2). NucleoType® Mouse PCR, NucleoType® Blood PCR and NucleoType® Plant PCR and NucleoType® Seed PCR already contain appropriate amounts of NucleoType® HotStart PCR Master Mix.

Product	Specifications	Preps	REF
NucleoType® DirectLyse Reagent	Lysis reagent for simple PCR-ready DNA extraction allowing direct utilization in subsequent PCR or qPCR reactions; contains extraction buffer XLR and Liquid Proteinase K	≤ 500	743220
NucleoType® Mouse PCR	For direct genotyping from mouse samples (tail clips, ear punches, hair follicles, blood); contains Lysis Buffer M, Liquid Proteinase K and NucleoType® HotStart PCR Master Mix (2x)	25 / 100 / 500	743200.25 / .100 / .500
NucleoType® Blood PCR	For direct genotyping from blood samples; contains Blood Transfer Tool (BTT), Inhibitor Removal Pearls (IRP) and NucleoType® HotStart PCR Master Mix (2x)	25 / 100 / 500	743201.25 / .100 / .500
NucleoType® Plant PCR	For direct plant genotyping; contains Plant Transfer Tool (PTT) and NucleoType® HotStart PCR Master Mix (2x)	25 / 100 / 500	743202.25 / .100 / .500
NucleoType® Seed PCR	For direct seed genotyping; contains Lysis Buffer P, Liquid Proteinase K and NucleoType® HotStart PCR Master Mix (2x)	25 / 100 / 500	743203.25 / .100 / .500

2.2 Product specifications

The incorporated hot start Taq polymerase can perform consistently well on a broad range of templates, including both GC and AT rich. The buffer system allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions. The mix is notably resistant to PCR inhibitors. The mix is suitable for direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood, tissue and food samples like cheese, milk and meat.

The NucleoType® HotStart PCR Master Mix has an error rate of circa 1 error per 2.0×10^5 nucleotides incorporated. PCR producs generated are A-tailed and may be cloned into TA cloning vectors.

2.3 Remarks regarding quality control

In accordance with MACHEREY-NAGEL's Quality Management System, each component of the NucleoType® DirectLyse Reagent is tested against predetermined specifications to ensure consistent product quality.

3 Storage conditions

The NucleoType® HotStart PCR Master Mix should be stored upon arrival at +4 °C or -20 °C. The mix is stable for at least 12 month when stored at this temperature. The mix 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 the mix at +4 °C or -20 °C. 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

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 Colony PCR

Prepare a reaction with a total volume of 10 µL. Using tools like a pin, needle, or toothpick, lightly touch the surface of a bacterial colony and promptly transfer it into the PCR reaction mixture (one-touch only, approximately 1 second, without stirring). Utilize the hot start technology of this product to set up the reaction at room temperature (18 – 25 °C).

Note: When setting up colony PCR with a 10 µL reaction volume, ensure to transfer an adequate amount (very minimal) while minimizing excess. It is advisable to use tools with tiny tips like pins, needles, or toothpicks. Avoid using wooden toothpicks as they may transfer excessive sample material or absorb the reaction mixture. Additionally, pipette tips may also transfer excessive material if bacterial material enters the tip.

Set up the colony PCR mixture as follows

	10 µL	25 µL	50 µL
NucleoType® HotStart PCR Master Mix (2x)	5 µL	12.5 µL	25
Forward primer	2 µL primer (stock concentration 1 µM or 1 pmol/µL)	5 µL primer (stock concentration 1 µM or 1 pmol/µL)	10 µL primer (stock concentration 1 µM or 1 pmol/µL)
Reverse primer	2 µL primer (stock concentration 1 µM or 1 pmol/µL)	5 µL primer (stock concentration 1 µM or 1 pmol/µL)	10 µL primer (stock concentration 1 µM or 1 pmol/µL)
DNA template	See recommendations above	See recommendations above	See recommendations above
H_2O	To 10 µL	To 25 µL	To 50 µL

Reaction setup for single-plex 10 µL PCR

The 10 µL reaction is the recommended standard reaction volume for the NucleoType® HotStart PCR Master Mix. 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 DNA template
  → 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

5.2 Genotyping other PCR reactions

For tissue PCR, adhere to the guidelines outlined for colony PCR when conducting direct PCR. Alternatively, for increased flexibility in sample material range and suitability, consider utilizing the NucleoType® DirectLyse Reagent (REF 743220; refer to ordering information).

For **foodstuffs such as cheese, milk, and meat**, adhere to recommendations for colony PCR.

For **genotyping of tail clippings, ear punches, blood, and hair**, we recommend to use the NucleoType® Mouse PCR kit (REF 743200; see ordering information).

For **genotyping of blood samples**, we recommend to use the NucleoType® Blood kit (REF 743201; see ordering information).

To facilitate plant genotyping, we recommend utilizing the NucleoType® Plant kit (REF 743202; please refer to ordering information). Similarly, for seed genotyping, we recommend employing the NucleoType® Seed kit (REF 743203; please refer to ordering information).

5.3 PCR cycling parameters

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)	

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 nor for quantitative PCR!

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

*: The optimal annealing / extension time is primer dependent. Primer with melting temperature above 60 °C are recommended.
Optimal annealing tempearture 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.

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 e.g. 1 % agarose gel 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	• 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. *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 extract in PCR.* • Make sure to use at most 1 µL lysate as template per reaction. *Liquid Proteinase K was skipped or heat incubation step was skipped.* • 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. *PCR cycling conditions not optimal.* • Decrease annealing temperature. Test different primer annealting temperatures. Increase extension time. Increase number of cycles up to 40.
Too little amplicon yield	• Try to adjust annealing temperature and extension time
Amplicon does not have the correct size	• 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	• 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 / Related products

Product	REF	Pack of
NucleoType® DirectLyse Reagent	743220	up to 500 extractions
HotStart PCR Master Mix	743215	2.5 mL
NucleoType® Mouse PCR	743200.25 / .100 / .500	25 / 100 / 500 reactions
NucleoType® Blood PCR	743201.25 / .100 / .500	25 / 100 / 500 reactions
NucleoType® Plant PCR	743202.25 / .100 / .500	25 / 100 / 500 reactions
NucleoType® Seed PCR	743203.25 / .100 / .500	25 / 100 / 500 reactions
Liquid Proteinase K	740396	5 mL
Liquid Proteinase K	740396.30	30 mL

Visit ***www.mn-net.com*** for more detailed product information

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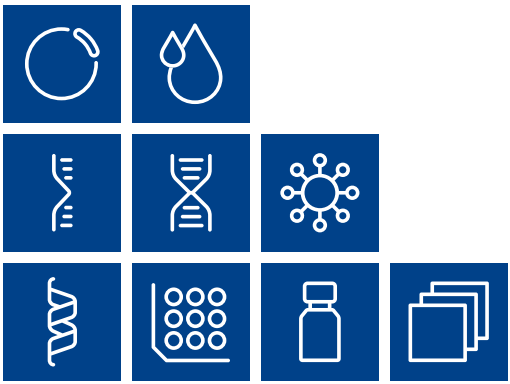
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-333
support@mn-net.com



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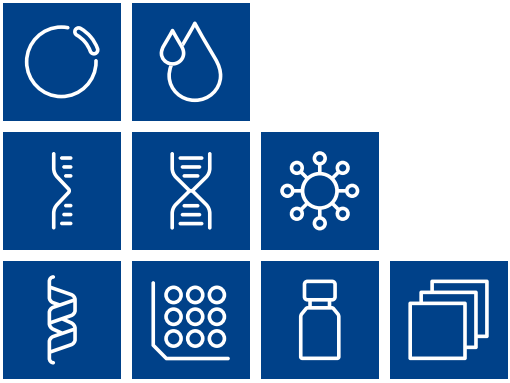

MACHEREY-NAGEL

www.mn-net.com

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