

User manuals

**NucleoType® HotStart PCR
Master Mix****743215**

Dear valued customer,

Thank you for choosing MACHEREY-NAGEL for your application. We have attached a short protocol for your review.

To obtain the best results we recommend to follow the detailed protocol available online, especially when you are a first time user of this kit.

All important links to the above mentioned products are listed in this leaflet.

Important information regarding product components, specifications, safety instructions, and processing protocols, can all be found on the product website and accessed easily via the QR code.

QR-Code product website



[qr.mn-net.com/qr/\(241\)743215](https://qr.mn-net.com/qr/(241)743215)

Use the following QR code or the link below for direct access to the user manual.

QR-Code user manual



[qr.mn-net.com/qr/\(IFU\)743215](https://qr.mn-net.com/qr/(IFU)743215)

It is strongly recommended to read the detailed protocol section of the user manual if using the kit for the first time. However, experienced users may refer to the protocol at a glance. The protocol at a glance is designed to be used only as a supplemental tool for quick reference while performing the purification procedure.

We are constantly improving our products and we reserve the right to make changes or additions to protocols. Please check for updated revisions for previously downloaded manuals.

This leaflet does not replace the full manual!

NucleoType HotStart PCR Master Mix

The NucleoType HotStart PCR Master Mix is recommended to be used either for colony PCR reactions or in combination with one of the following NucleoType products:

NucleoType Mouse PCR, NucleoType Blood PCR and NucleoType Plant PCR and NucleoType Seed PCR already contain appropriate amounts of the 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 / 743200.100 / 743200.500
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 / 743201.100 / 743201.500
NucleoType® Plant PCR	For direct plant genotyping; contains Plant Transfer Tool (PTT) and NucleoType® HotStart PCR Master Mix (2x)	25 / 100 / 500	743202.25 / 743202.100 / 743202.500 /
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 / 743203.100 / 743203.500 /

Recommendation for 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.

Product	10 µL	25 µL	50 µL
NucleoType® HotStart PCR Master Mix (2x)	5 µL	12.5 µL	25 µL
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 recommendation above	See recommendation above	See recommendation above
H ₂ O	To 10 µL	To 25 µL	To 50 µL

A final concentration of 0.2 µM per primer is recommended.

PCR cycler program conditions

PCR cycler program conditions			
Initial Denaturation	95 °C		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

The PCR cycler program parameters, including temperature and time, depend upon the specific requirements of your PCR and primer conditions. Customize these parameters according to your specific needs and refer to the user manual for additional guidance and information on optimization. The NucleoType® HotStart PCR Master Mix contains a pink dye for tracking during agarose gel electrophoresis. No additional tracking color or glycerol has to be added.

QR-Code Safety Data Sheet

Please find a digital version of the safety data sheets by following the link below:



www.mn-net.com/sds



We strongly recommend to carefully read the detailed protocol section of the product's user manual. If you have any questions about the protocol or product, please contact our Technical Support.

Contact MN

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