NucleoType Seed PCR

Storage conditions and preparations of working solutions

The NucleoType Seed PCR kit should be stored upon arrival at +4 °C or -20 °C (recommended).

The kit is stable for at least 12 months when stored at this temperature. The kit can be shipped at ambient temperature (18–25 °C) for up to 3 months. Short time exposure (up to 14 days) at temperatures up to 37 °C is tolerable.

Store all kit components at +4 °C or -20 °C (recommended) upon arrival and after first time usage. 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.

Lysis Buffer P, Liquid Proteinase K, and NucleoType HotStart PCR Master Mix (2x) are ready to use.

Prepare a primer mix according to the recommended concentration per primer as described.

Kit contents

	NucleoType Seed PCR		
REF	25 preps 743203.25	100 preps 743203.100	500 preps 743203.500
Lysis Buffer P	12 mL	60 mL	250 mL
Liquid Proteinase K	50 µL	250 µL	1250 µL
NucleoType HotStart PCR Master Mix (2x)	125 µL	500 µL	2 x 1250 µL
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Reagents, consumables, and equipment to be supplied by user

Reagents

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

Consumables

- Disposable pipette tips
- PCR tubes

Equipment

- Manual pipettes
- Thermoshaker or vortexer
- Personal protection equipment (lab coat, gloves, goggles)
- PCR cycler
- Gel electrophoretic equipment or Bioanalyzer® for analysis of generated amplicons

About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoType Seed PCR** kit before using these products.

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.

Product description

The basic principle

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

The **NucleoType Seed PCR** kit is designed for rapid plant typing experiments using seed material as sample, without the need to purify DNA.

The **NucleoType Seed PCR** kit provides the optimized Lysis Buffer P and Proteinase K for a simple sample preparation within a few minutes.

Kit specifications

Kit specifications at a glance	
Parameter	**NucleoType Seed PCR**
Technology	Simple sample preparation suitable for hot start PCR
Format	10 µL HotStart PCR (optional up to 50 µL)
Sample type	Hard plant material like, e.g., seeds from soybeen, wheat, corn, rice, and tobacco as well as from moss, fern leaf, and fir needle
Preparation time	Simple sample preparation in less than 5 min; PCR cycling: 30–90 min (cycler and target size dependent)
Amplicon size	Up to 2000 bp
Analysis	Gel electrophoresis: Approx. 30 min (40 samples); Bioanalyzer®: Approx. 40 min (12 samples)

Handling, preparation, and storage of starting material

The kit is designed to perform genotyping from fresh seed material. Plant material stored at 0–8 °C for several days or frozen material may also be used. However, kit performance with plant material stored for a long time might differ to the performance of fresh material.

Lysis, disruption, and transfer of sample material

In order to obtain reliable plant typing data, it is important to obtain a sufficient amount of DNA in a form suitable to serve as template for subsequent PCR amplification.

The **NucleoType Seed PCR** kit contains Lysis Buffer P and Proteinase K enabling a simple sample preparation within in few minutes for, e.g., plant seeds. The lysis procedure releases DNA in sufficient amount and quality to serve as template for the subsequent PCR.

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, target length, and PCR cycler setup. For several primer pairs with T_m ranging from 40–75 °C the following PCR programs have been used successfully.

PCR program 1 (three step program for typical endpoint PCR cycler)			
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. 60–70 min (total run time is annealing / elongation temperature and machine dependent)	

Note: For amplification of fragments smaller 1000 bp and / or for amplifications with a PCR machine with slow ramp rates (2 °C/second) the annealing time and extension time may be reduced stepwise, e.g. down to 15 seconds for annealing and 15 seconds for extension.

PCR program 3 (e.g., LightCycler® 1.5 in glass 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 (total run time is annealing / elongation temperature and machine 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.

Analysis of PCR products

The PCR products (amplicons) can directly be analyzed using 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 an e.g., 1–2 % 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.

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

** For initial testing an annealing / extension time of 20 and/or 60 seconds is recommended!

*** 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.

Protocols

Plant typing of seeds using Lysis Buffer P and Proteinase K

1 **Prepare final Lysis Buffer P**

Add **100–500 µL Lysis Buffer P** into a lysis tube (1.5 mL or 2.0 mL; not provided).

Add **0.5 µL Liquid Proteinase K** per 100 µL Lysis Buffer P.

Note: If several samples are processed at a time, a premix of Lysis Buffer P and Liquid Proteinase K can be prepared. Such a premix is stable at least 1 h at room temperature (18–25 °C).

2 **Incubate sample and release DNA**

Place a plant seed sample into the final lysis buffer.

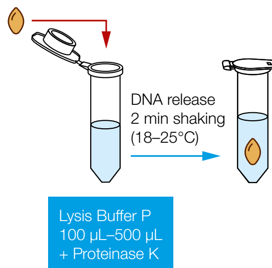
Recommendations for adequate final lysis buffer volume

- Small seeds like *Arabidopsis*, wheat, rice: 100 µL Lysis Buffer P
- Medium seeds like soybeen: 200 µL Lysis Buffer P
- Large size seed like corn or cotton: 500 µL Lysis Buffer P

Incubate 2 min shaking at room temperature (18–25 °C; DNA release step).

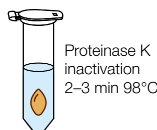
Note: If continuous shaking is not possible, a motionless incubation can be performed. However, an initial shaking is required.

Note: Incubation times of 1–5 min are possible and show similar results.



3 **Deactivate Proteinase K**

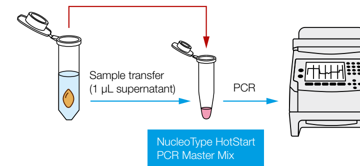
Incubate 2–3 min at 98 °C (Proteinase K inactivation step) while shaking. If continuous shaking is not possible, a motionless incubation can be performed.



4 **Transfer sample into the PCR**

Use 1 µL lysate as template for subsequent PCR.

Note: For storage of the lysate, the plant seed has to be removed from the lysate! The lysate can then be stored up to 6 months at +4° C or -20 °C. Even storage at room temperature (18–25 °C) is possible for up to 6 weeks. Repeated freeze-thaw cycles are unproblematic.



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

A final PCR with 10 µL volume is the recommended standard reaction volume for the **NucleoType Seed PCR** kits. Due to the hot start technology of this product, the reaction setup 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 target 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 plant sample directly from the Plant Transfer Tool (PTT) or 1 µL from the seed lysate.

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 PCR volume

- *Reduction of initial 10 µL PCR volume during cycling*
  Depending on the PCR tube size and quality, the initial 10 µL setup volume might shrink to approximately 8 µL during cycling. This is acceptable and does not impair typing performance. If volume reduction is more pronounced, use a smaller and / or tighter reaction tube.

To little or no amplicon detected

- *Unfavorable primer selection*
  Make sure that the primers 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.
- *Unfavorable PCR program*
  Try to adjust annealing temperature and time as well as extension time. Note that PCR machines with rapid ramp rates require longer annealing and extension times than PCR machines with slow ramp rates, because there is less amplification time for the polymerase during ramping!

Amplicon does not have the correct size

- *Primer selection*
  Make sure that the primers are selected well and are able to amplify the desired target from 1–10 ng or purified genomic DNA.

Amplicon number is not correct

- *Sensitivity of analysis method*
  Make sure that the analysis method has enough resolving power to discriminate the two different sizes of DNA fragments.
- Use Bioanalyzer® instead of gel electrophoreses 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).

Product use and restriction / warranty

NucleoType Seed 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.

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

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