Bioanalysis

MACHEREY-NAGEL

User manual

Lysis reagent for direct PCR and direct qPCR

■ NucleoType DirectLyse Reagent

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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

Table of contents

1 Components

1.1 Kit contents

	NucleoType DirectLyse Reagent
REF	**743220 (up to 500 extractions)**
Extraction Buffer XLR	60 mL
Liquid Proteinase K	250 µL

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

Reagents

- PCR / qPCR reagents for follow-up analysis

Consumables

- Disposable pipette tips (aerosol barrier pipette tips are recommended to avoid cross-contamination)
- Microcentrifuge tubes (1.5 or 2.0 mL)

Equipment

- Manual pipettors
- Heat incubator
- Vortex mixer
- Centrifuge for microcentrifuge tubes (1.5 or 2 mL)
- Personal protection equipment (lab coat, gloves, goggles)
- PCR cycler for follow-up analysis

1.3 About this user manual

It is strongly recommended to read the instructions of this user manual carefully before using this product. All technical literature is also available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

2 Product description

2.1 The basic principle – intendet use

The **NucleoType DirectLyse Reagent is** a reagent set for simple DNA extraction without column-based isolation steps.The extracted DNA can be used for subsequent PCR or qPCR analysis. NucleoType DirectLyse Reagent is well-suited for various sample materials, including ear punches (bovine, pig, mouse,…), blood, hair, hair follicles, and swabs. While lysis of other sample types may be possible, it requires validation by the customer. Sample material is incubated with a mix of Extraction Buffer XLR and Liquid Proteinase K for a few minutes at room temperature followed by a brief incubation at 99 °C. Thereafter, an aliquot of extracted DNA may be used as template for PCR / qPCR analysis without further clean-up steps. The procedure releases DNA and eliminates / reduces PCR inhibitors from the sample.

The lysates are compatible with end point PCR and gel electrophoretic analysis, as well as with real time PCR analysis methods, including SYBR Green and probe (e.g. TaqMan) readouts.

The PCR analysis can be applied for genotyping, transgenic identification, identity testing, multiplex PCR, melt point analysis, as well as detecting bacterial and viral DNA in samples.

2.2 Product specification

Table 1: Kit specifications at a glance

Parameter	NucleoType DirectLyse Reagent
Technology	Lysis reagent for direct PCR / qPCR
Format	Column-free lysis reagent for manual use
Sample material	Various biological sample materials such as ear punches, animal tissue, blood, hair, plant tissue, plant seeds, swab.
Sample amount*	few cells up to approx. 200 mg
Elution volume*	20 – 500 µL
Preparation time	10 – 15 minutes
Suitable downstream analysis	PCR, qPCR
Use	For research use only

*Optimal sample amount and reaction volume may vary greatly depending on sample material and downstream PCR and qPCR reaction.

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.

2.4 Storage conditions and preparation of working solution

Before first use, the NucleoType DirectLyse Reagent can be shipped and stored at ambient temperature (15 – 25 °C) and is stable until: see package lable.

All buffers and components are delivered ready-to-use.

After first use, store the components from NucleoType DirectLyse Reagent at -20 °C or at 2 – 8 °C for up to one year.

A master premix of NucleoType DirectLyse Reagent, consisting of Extaction Buffer XLR and Liquid Proteinase K, (see protocols) should be used immediately or stored aliquoted at -20 °C for up to one year.

3 Safety instructions

When working with the **NucleoType DirectLyse Reagent** wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

The waste generated with the **NucleoType DirectLyse Reagent** has not been tested for residual infectious material. Depending on the source and nature of the sample, it is essential to treat liquid waste as potentially infectious. Proper handling and disposal should be carried out in accordance with local safety regulations.

3.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

4 Protocols

The following information and protocols are based on recommendations that have been biologically tested and verified under given conditions. The protocol may be optimized depending on the sample material, sample quantity, and subsequent assay. For successful PCR or qPCR analysis it is important to choose a suitable amount of sample material and volume of NucleoType DirectLyse Reagent in order to release a sufficient amount of DNA and to avoid negative effects through PCR inhibitors originating from the sample.

Before starting the preparation:

- Check if all materials according to section 1.2 are available.

4.1 Extraction protocols for ear punches, cultivated cells, or blood

Procedure

1	**Transfer sample material** (recommended sample amount:. e.g. 1 mm diameter ear punch, max. 1 × 10^6 HeLa cells, or 2 µL blood) into a 1.5 mL or 2 mL tube. *Note: Ear punches can vary significantly in diameter and thickness depending on the animal species and the type of punch used. Small sample amounts can be benefical to extraction efficiency and downstream analysis.*
2	**Add 100 µL Extraction Buffer XLR.**
3	**Add 0.5 µL Liquid Proteinase K.** *Note: Typically, a volume of 0.5 µL Liquid Proteinase K is sufficient. In some cases it might be beneficial to use up to 2.5 µL Liquid Proteinase K.* *Note: If seveal samples are processed, a premix may be prepared, e.g. for 10 samples: 1 mL Extraction buffer XLR and 5 µL Liquid Proteinase K. The mixture is stable at room temperature for up to 1 h. Store the mixture aliquotted at -20 °C for later use.*
4	**Mix by pipetting.**
5	**Incubate at room temperature (15 – 25 °C) for 2 min.** *Note: DNA is released in this step.* *Note: Typically this short incubation is sufficient for adequate DNA release. It is not necessary to completely dissolve / lyse the sample. For some applications, a longer incubation might be beneficial.*
6	**Optional: Centrifuge for 3 s at 8,000xg if solution contacted the upper part of the tube or lid.** *Note: It is important, that all the solution / material is subjected to the following heat incubation. Ideally, use an incubator which heats the lid or make sure that all solution / material is in the lower part of the tube if using a heating block which does not heat the lid of the tube.*
7	**Incubate for 5 min at 99 °C.**

8 **Centrifuge for 2 min at 12,000 xg** in order to sediment any debris and to obtain a clear supernatant.

Note: Heat incubation and centrifugation eliminates / reduces PCR inhibitors from the solution.

Note: Typically, cleared lysates are stable for at least one day at room temperature (15 – 25 °C), several days at 4 °C, and two years at -20 °C. For quantitative analysis, immediate use or storage at -20 °C is recommended. Lysate stability has been evaluated with blood lysates; stability of lysates from other sample types may vary.

9 **Extracted DNA may be used as a template for PCR or qPCR without further clean-up steps. We recommend to use 1 – 2 µL of the clear supernatant as template for a 20 µL PCR.**

Note: Given the variability in DNA concentration and PCR efficiency, users can perform a series of dilutions to ascertain the optimal concentration for their PCR. The input DNA may be increased or further diluted.

The NucleoType DirectLyse Reagent has been tested with the following PCR systems:

a. *MACHEREY-NAGEL NucleoType PCR Master Mix (endpoint PCR)*

b. *Thermo Scientific Maxima SYBR Green qPCR Master Mix (qPCR with SYBR Green readout)*

c. *PCR Biosystems 2x qPCRBIO SyGreen Blue Mix Separate-ROX (qPCR with SYBR Green readout)*

d. *Bioline / Meridain SensiFAST Probe Lo-ROX Kit (qPCR with TaqMan probes readout)*

e. *Thermo Fisher Quantifiler™ Human DNA Quantificationr Kit*

f. *Qiagen QuantiTect Multiplex PCR Kit*

4.2 Protocol for processing swabs

Procedure

1 **Transfer the swab tip into a 2 mL tube** (screw cap tube is recommended). Cut or break off the swab shaft.

2 **Add 500 µL Extraction buffer XLR.**

Note: Make sure to use a sufficient volume of Extraction buffer XLR to cover the swab tip. Depending on swab size, volume of the reagent has to be adjusted.

3 **Add 0.5 µL Liquid Proteinase K** and close the tube.

4 **Mix.**

5 **Incubate at room temperature** (15 – 25 °C) for 15 min.

Note: DNA is released in this step.

6 Optional: Centrifuge for 3 s at 8,000 xg if solution contacted the upper part of the tube or lid.

7	**Incubate for 10 min at 99 °C.**
8	**Let the tube cool down.**
9	**Centrifuge for 2 min at 12,000 xg** in order to sediment any debris and to obtain a clear supernatant. *Note: Heat incubation and centrifugation eliminates / reduces PCR inhibitors from the solution.*
10	Use 1 – 2 µL of the clear supernatant as template for a 20 µL PCR. *Note: It is recommended to vortex thoroughly and briefly centrifuge to clean the lid before withdrawl of an aliquot.* See section 4.1 for further notes.

4.3 Protocol recommendations for various sample types

Procedure

1. Select the sample amount and Extraction buffer XLR volume thoughtfully to liberate the required amount of DNA while minimizing the presence of PCR inhibitors in the sample. The suitable quantities may differ significantly depending on the sample types and the PCR system utilized for analysis. Refer to the following table for guidance.

Table 1: Recommendations for choosing appropriate sample amounts and Extraction buffer XLR volumes.

Sample type	Sample amount	Volume of Extraction buffer XLR	Comment
Mouse ear punch	one punch 1 mm Ø	20 µL	NucleoType Mouse PCR kit may be used alternatively for endpoint PCR analysis.
Mouse tail clip	Approximately 1 mm from the outer end of the tail	20 µL	dto.
Mouse hair	Approximately 3 – 30 fur hairs	20 µL	Make sure to place the hairs at the bottom of the tube, preferably roots down. Short centrifugation of the closed lysis tube may help to spin down the hairs. A slant tweezer with a 1 × 3 mm flat area to grab the fur hairs is recommended.

Blood	2 µL	100 µL	For direct PCR without simple sample preparation NucleoType Blood PCR kit is recommended. Avian and reptile blood requires much less blood due to nucleated red blood cells.
Cattle ear punch	one punch 1 mm Ø, ~ 1 – 4 mg	100 µL	Use of ear punches from calfs is recommended. Depending on ear size, use only one skin layer.
Pig ear punch	one punch 1 mm Ø, ~ 1 – 4 mg	100 µL	Use of ears punches from piglets is recommended. Depending on ear size, use only one skin layer.
Swab	1 medium size	500 µL	e.g. Copan Floq-Swabs (4520CS01)
Tiny seeds like tobacco, Arabidopsis	1 kernel (~0.01 – 0.1 mg)	20 µL	NucleoType Seed PCR kit may be used alternatively for endpoint PCR analysis.
Small seeds like wheat, rice	1 kernel (~10 – 100 mg)	100 µL	dto
Medium seeds like soybeen	1 kernel (~100 – 200 mg)	200 µL	dto
Large seeds like corn or cotton	1 kernel (~200 – 500 mg)	500 µL	dto.
Cultivated cells (e.g. HeLa cells)	100 – 1.000.000 cells	100 µL	-
Plant leaf	one punch 1 mm Ø	200 µL	For direct PCR without simple sample preparation the NucleoType Plant PCR kit is recommended. Depending on the thickness of the leaf, lysis buffer volume or punch diameter has to be adjusted.

2. **Transfer the sample into a 1.5 or 2 mL tube.**

3. **Add the chosen volume of Extraction buffer XLR into the tube.**

4. **Add 0.5 µL Liquid Proteinase K.**

Note: Typically, a volume fo 0.5 µL Liquid Proteinase K is sufficient. In some cases it might be beneficial to use up to 2.5 µL Liquid Proteinase K.

5. **Mix by pipetting / vortexing.**

6. **Incubate at room temperature (15 – 25 °C) for 2 min.**

Note: DNA is released in this step.

Note: Typically this short incubation is sufficient for adequate DNA release. It is not necessary to completely dissolve / lyse the sample. For some applications, a longer incubation might be beneficial.

7. Optional: Centrifuge for 3 s at 8,000 xg if solution contacted the upper part of the tube or lid.

Note: It is important, that all the solution / material is subjected to the following heat incubation. Ideally, use an incubator which heats the lid or make sure that all solution / material is in the lower part of the tube if using a heating block which does not heat the lid of the tube.

8. **Incubate for 5 min at 99 °C.**

9. **Centrifuge for 2 min at 12,000 xg** in order to sediment any debris and to obtain a clear supernatant.

Note: Heat incubation and centrifugation eliminates / reduces PCR inhibitors from the solution.

Note: Typically, cleared lysates are stable for at least one day at room temperature (15 – 25 °C), several days at 4 °C, and two years at -20 °C. For quantitative analysis, immediate use or storage at -20 °C is recommended. Lysate stability has been evaluated with blood lysates; stability of lysates from other sample types may vary.

10. **Use 1 – 2 µL of the clear supernatant as template for a 20 µL PCR.**

See section 4.1 for further notes.

5 Appendix

5.1 Troubleshooting

Problem	Possible cause and suggestions
No PCR signal	• Use less sample material or more Extraction Buffer XLR. Make sure that all the solution is on the bottom of the tube during heat treatment in order to avoid insufficient heat treatment for material sitting in the lid. • Unfavorable primer selection: Make sure that primers are selected well and are able to amplify the desired target DNA. Test different primer annealing temperatures.
PCR cycling conditions not optimal.	• Decrease annealing temperature. Test different primer annealing temperatures or Increase extension time.
Partial PCR inhibition – no linearity in qPCR within a dilution series	• If you intend to employ real-time PCR for target quantification (qPCR), it is recommended to conduct a linearity assessment through a dilution/inhibition test. Utilize, for instance, 1 µL, and 1:2, and 1:4 dilutions of the lysate, and check for the emergence of a delay of approximately 1 cycle per 1:2 dilution. If you observe earlier Cp values resulting from dilution, it indicates partial PCR inhibition. In such cases, consider using reduced sample material and/or a higher volume of Extraction Buffer XLR for sample lysis.

5.2 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.

6 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.

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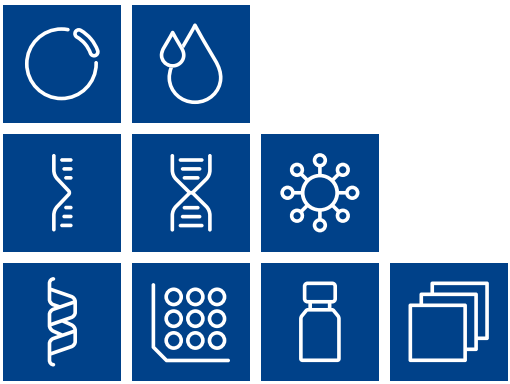
Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 (0) 24 21 969-333
support@mn-net.com



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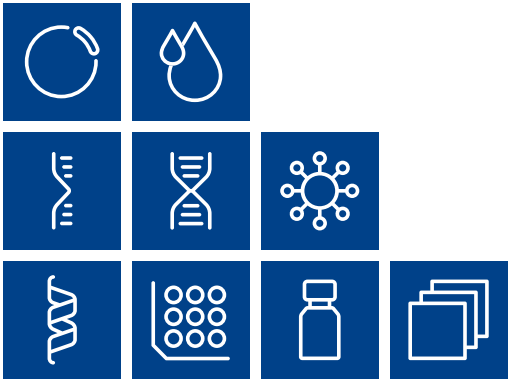

MACHEREY-NAGEL

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