Genomic DNA from microoganisms

User manual

NucleoSpin® Microbial DNA

August 2015 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

Genomic DNA from microorganisms

Protocol-at-a-glance (Rev. 01)

		NucleoSpin® Microbial DNA
1 Prepare sample		< 40 mg microbial pellet (wet weight) 100 µL BE
2 Sample lysis	 	Transfer sample in NucleoSpin® Bead Tube Type B 40 µL Buffer MG 10 µL Liquid Proteinase K Agitate on a swing mill or similar device 4–12 min 11,000 x *g*, 30 s
3 Adjust binding conditions	 	600 µL Buffer MG Vortex 3 s 11,000 x *g*, 30 s
4 Bind DNA	 	Load 500–600 µL sample on NucleoSpin® Microbial DNA Column 11,000 x *g*, 30 s
5 Wash silica membrane	 	**1st** 500 µL BW — 11,000 x *g*, 30 s **2nd** 500 µL B5 — 11,000 x *g*, 30 s
6 Dry silica membrane	 	11,000 x *g*, 30 s
7 Elute DNA	 	100 µL BE RT, 1 min 11,000 x *g*, 30 s

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1 Components

1.1 Kit contents

	NucleoSpin® Microbial DNA	
REF	**10 preps 740235.10**	**50 preps 740235.50**
Lysis Buffer MG	10 mL	38 mL
Wash Buffer BW	6 mL	30 mL
Wash Buffer B5 (Concentrate)*	6 mL	6 mL
Elution Buffer BE**	13 mL	30 mL
Liquid Proteinase K	120 µL	600 µL
NucleoSpin® Bead Tubes Type B	10	50
NucleoSpin® Microbial DNA Columns (light green rings)	10	50
Collection Tubes (2 mL)	20	100
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* For preparation of working solutions and storage, see section 3.

** Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

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

Reagents

- 96–100 % ethanol

Consumables

- 1.5 mL or 2 mL microcentrifuge tubes for microbial sample sedimentation
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Sample disruption device: swing mill or similar device (e.g., Schwingmühle MM200, MM300, MM400 (Retsch®); FastPrep® System (MP-Biomedicals); Precellys® (Bertin Technologies); MagNA Lyser (Roche); TissueLyser (QIAGEN); Bullet Blender® (Next Advance); Mini-Beadbeater (Biospec Products); Speed Mill (Analytik Jena); Vortex Adapter for Vortex-Genie® 2 X (MoBio))
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Microbial DNA** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is 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 revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® Microbial DNA** kit is designed for efficient isolation of genomic DNA from microbial samples. DNA can be isolated from a wide variety of microorganisms such as gram-negative, and gram-positive bacteria as well as yeasts, e.g., *Escherichia coli, Bacillus subtilis, Corynebacterium glutamicum, Saccharomyces cerevisiae.* Preparation of the collected samples containing the microbes of interest should be in pellet format.

Preliminary data also indicate the usability of the kit for DNA isolation from fungal mycelia, e.g., *Aspergillus nidulans*, from bacterial spore suspensions, e.g., *Geobacillus stearothermophilus*, and from plant pollen, e.g., honey bee pollen baskets. For optimal DNA yield, bead tubes different from the ones included in the kit might be required for such applications (see section 2.4).

Microbial samples such as gram-positive bacteria, yeast, and spores can be difficult to lyse due to their strong complex cell wall structures. The NucleoSpin® Microbial DNA kit replaces enzymatic lysis by utilizing mechanical disruption of cell wall structures with the NucleoSpin® Bead Tubes. The NucleoSpin® Bead Tubes can be used in combination with many compatible disruptive devices (see section 2.4.1). High DNA yields can be obtained with the NucleoSpin® Bead Tubes from a large variety of sample types – enabling the procedure to be convenient, fast, and easy. Alternative bead types can be ordered separately for select sample types (see section 2.4.3 for recommendations).

2.2 Kit specifications

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Microbial DNA
Technology	Silica-membrane technology
Format	Mini spin column
Sample material	Microbial cell culture pellets of gram-positive and gram-negative bacteria, yeasts
Sample amount	Up to approx. 40 mg wet weight
Typical yield	Varies by sample and disruption device. 5–25 µg DNA from approx. 30 mg wet weight microbial pellet can be obtained.
A_{260}/A_{280}	1.6–2.0
Elution volume	100–200 µL
Preparation time	35 min/6 preps
Binding capacity	60 µg

2.3 Handling, preparation, and storage of starting materials

Cells should be harvested from fresh microbial cultures by sedimentation via centrifugation. Supernatant should be removed by aspiration. Microbial cell pellets can be used fresh or stored at -20 °C to -80 °C before starting DNA isolation.

2.4 Lysis and disruption of sample material

In order to obtain optimal yields of DNA from sample material, a complete disruption of the sample material is necessary. Sample disruption efficiency depends on the following parameters and can be achieved by following suggestions outlined in the subsequent sections.

2.4.1 Sample type and disruption device

Sample and disruption device are to be supplied by user.

The following devices are compatible with NucleoSpin® Bead Tubes:

- Schwingmühle MM200, MM300, MM400 (Retsch®)
- FastPrep® System (MP-Biomedicals)
- Precellys® (Bertin Technologies)
- MagNa Lyser (Roche)
- TissueLyser II and Tissue Lyser LT (QIAGEN)
- Bullet Blender® (Next Advance)
- Mini-Beadbeater (Biospec Products)
- Speed Mill (Analytik Jena)
- Vortex Adapter for Vortex-Genie® 2 X (MoBio)

2.4.2 Lysis buffer composition, sample amount, volume of lysate, and temperature

Lysis buffer, maximal sample amount, and volume of liquid in the bead tube (sample amount + water + lysis buffer + Proteinase K) are specified in the corresponding NucleoSpin® kit. Room temperature (18–25 °C) is recommended as the working temperature.

2.4.3 Type of bead tube, time of disruption, and frequency of disruption

Bead type, disruption time, and frequency / speed must be optimized for a given sample for maximal results of DNA yield and quality.

- **Type of bead tube**

NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads)
Recommended for soil and sediment (included in NucleoSpin® Soil, see ordering information, section 7.2).

NucleoSpin® Bead Tubes Type B (40–400 µm glass beads)
Recommended for gram positive and negative bacteria (included in NucleoSpin® Microbial DNA, see ordering information, section 7.2).

NucleoSpin® Bead Tubes Type C (1–3 mm corundum)
Recommended for yeast (see ordering information, section 7.2).

- **Time and frequency of disruption**

The following recommendations have been established for a Retsch Schwingmühle MM300 operating at highest frequency (30 Hertz). **For using other disruption devices, and other sample materials time and frequency have to be optimized.**

Sample material	NucleoSpin® Bead Tube	Disruption time
Gram-negative bacteria *E.g., Escherichia coli, Vibrio fischeri*	NucleoSpin® Bead Tubes Type B (Alternative: Type A, Type C)	4 min
Gram-positive bacteria *E.g.,* Bacillus subtilis, Corynebacterium glutamicum	NucleoSpin® Bead Tubes Type B (Alternative: Type A)	12 min
Yeast *E.g., Saccharomyces cerevisiae*	NucleoSpin® Bead Tubes Type C	12 min
Filamentous fungi *E.g., Aspergillus spec., Rhizopus spec.*	NucleoSpin® Bead Tubes Type C	12 min

Note: Performance and stability testing has been conducted on the NucleoSpin® Bead Tubes A, B, and C on a Retsch® Schwingmühle MM300 at highest frequency (30 Hertz) for up to 15 minutes for optimal sample disruption, avoidance of DNA fragmentation, and tube durability. Other disruption devices (see section 2.4.1) will require different settings regarding frequency and duration for optimal performance with the selected sample material. Please note that the position of the tube within the machine (Retsch® Schwingmühle) is important for optimal performance! Please consult instruction manual of the machine.

WARNING:** Many modern disruption devices can cause very high energy input in bead tubes. Depending on bead tube type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause breaking up of the bead tubes!* ***It is the responsibility of the user to perform initial stability test for the used bead tubes under the conditions used! *Perform initial test with water instead of lysis buffer and moderate machine setting (low frequency, short time) in order to avoid spillage of chaotropic lysis buffer in case of tube breakage.*

2.5 Elution procedures

In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.

- **Convenient elution (standard elution)**: For convenience, elution can be performed by one time addition of 100 µL elution buffer onto the column.
- **High yield**: Two serial elutions of 100 µL each for total elution volume of 200 µL.
- **High concentration**: Use initial 100 µL eluate for second elution – 100 µL total elution volume, 2 elutions.

3 Storage conditions and preparation of working solutions

Attention:

Lysis MG and Wash Buffer BW contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers MG and BW contain chaotropic salts which can form highly reactive compounds when combines with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waster!

- All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.

Before starting any **NucleoSpin® Microbial DNA** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96–100 %) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer B5 can be stored at room temperature (18–25 °C) for at least one year.
- **Liquid Proteinase K** is ready to use. After first time use, store **Liquid Proteinase K** at 4 °C or -20 °C.

	NucleoSpin® Microbial DNA	
REF	**10 preps** **740235.10**	**50 preps** **740235.50**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	6 mL Add 24 mL ethanol

4 Safety instructions

The following components of the **NucleoSpin® Microbial DNA** kits contain hazardous contents.
Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.
Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
BW	Guanidine hydrochloride 36–50% + 2-propanol 20–50% *Guanidinhydrochlorid 36–50%* + 2-Propanol 20–50% CAS 50-01-1	 WARNING *ACHTUNG*	226, 302, 319, 336	210, 233, 264, 280, 301+312, 305+351+338, 330, 337+313, 370+378, 403+235
MG	Guanidinium thiocyanate 30–60 % *Guanidinthiocyanat 30–60 %* CAS 593-84-0	 WARNING *ACHTUNG*	302, 412, EUH031	260, 273, 301+312, 330
Proteinase K	Proteinase K, liquid 1–3 % *Proteinase K flüssig 1–3 %* CAS 39450-01-6	 WARNING *ACHTUNG*	317	261, 272, 280, 302+352, 333+313, 363

Hazard phrases

H226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*
H319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H336	May cause drowsiness or dizziness. *Kann Schläfrigkeit und Benommenheit verursachen.*
H412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
P233	Keep container tightly closed. *Behälter dicht verschlossen halten.*
P260	Do not breathe dust/fume/gas/mist/vapours/spray. *Staub/Rauch/Gas/Nebel/Dampf/Aerosol nicht einatmen.*
P261	Avoid breathing dust/fume/gas/mist/vapours/spray. *Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.*
P264	Wash … thoroughly after handling. *Nach Handhabung … gründlich waschen.*
P272	Contaminated work clothing should not be allowed out of the workplace. *Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.*
P273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P280	Wear protective gloves/protective clothing/eye protection/face protection. *Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.*
P301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/ if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt/… anrufen.*
P302+352	IF ON SKIN: Wash with plenty of water/… *BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/… waschen.*
P305+351+338	IF IN EYES: Rinse cautiously with water for several minuts. Remove contact lenses, if present and easy to do. Continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
P330	Rinse mouth. *Mund ausspülen.*
P333+313	If skin irritation or rash occurs: Get medical advice/attention. *Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.*
P337+313	If eye irritation persists: Get medical advice/attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.*
P363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*
P370+378	In case of fire: Use … to extinguish. *Bei Brand: … zum Löschen verwenden.*
P403+235	Store in a well-ventilated place. Keep cool. *An einem gut belüfteten Ort aufbewahren. Kühl halten.*

For further information please see Material Safety Data Sheets (***www.mn-net.com***).
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (**www.mn-net.com**).*


The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

5 Standard protocol for gram-positive and gram-negative bacteria

Before starting the preparation:

- Check if Buffer B5 was prepared according to section 3.
- Check section 2.4 for lysis and disruption of sample material.

1 Prepare sample

Harvest cells from a culture by centrifugation in a microcentrifuge tube (not provided). Discard supernatant.

Up to approximately 40 mg of wet weight microbial cell culture pellet can be used as sample material.

+ 100 µL Buffer BE

Add 100 µL Elution Buffer BE and resuspend cells.

Alternatively, high quality grade water (not provided) can be used.

2 Lyse sample

Transfer the cell suspension into the NucleoSpin® Bead Tube Type B (provided).

+ 40 µL Buffer MG

Add **40 µL Buffer MG**. Then, add **10 µL Liquid Proteinase K** and close the tube.

+ 10 µL Liquid Proteinase K

Note: It is not necessary to vortex here.

Agitate the **NucleoSpin® Bead Tube** on a swing mill or similar device.

Note: Optimal agitation duration, speed / frequency depends on the machine used. On a Retsch® Schwingmühle MM200, MM300, MM400, e.g., 4 min at maximal frequency (30 Hertz) is adequate for E.coli, 12 min for B.subtilis (see section 2.4). On the swing mill, position of the tube in the mill can considerably influence the result. Please consult the instruction manual of the device used.

Agitate

Centrifuge the NucleoSpin® Bead Tube **30 s** at **11,000 x g** to clean the lid.

Note: In this step foam is displaced from the screw cap, so that the cap can be removed in a clean way.

11,000 x *g*, 30 s

3 Adjust DNA binding conditions

Add 600 µL Buffer MG and mix (e.g, vortex for 3 s).

Note: Glass beads should be resuspended; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **30 s** at **11,000 x *g***.

Note: This centrifugation step is performed in order to clean the lid and sediment glass beads and cell debris.

+ 600 µL MG

Mix

11,000 x *g*, 30 s

4 Bind DNA

Transfer the supernatant (~500–600 µL) onto the NucleoSpin® Microbial DNA Column, placed in a 2 mL Collection Tube (provided).

Centrifuge for **30 s** at **11,000 x *g***. Discard collection tube with flow through. Put column into a fresh Collection Tube (2 mL, provided).

Load samples

11,000 x *g*, 30 s

5 Wash silica membrane

1st wash

Add **500 µL Buffer BW**. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

+ 500 µL BW

11,000 x *g*, 30 s

2nd wash

Add **500 µL Buffer B5** to the column and centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

+ 500 µL B5

11,000 x *g*, 30 s

6 Dry silica membrane

Centrifuge the column for **30 s** at **11,000 x *g*.**

Note: Residual wash buffer is removed in this step.

11,000 x *g*, 30 s

7 Elute highly pure DNA

Place the NucleoSpin® Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer BE** onto the column. Incubate at room temperature for 1 min. Centrifuge **30 s** at **11,000 x *g***.

For alternative elution procedures see section 2.5

+ 100 µL BE

RT, 1 min

11,000 x *g*, 30 s

6 Support protocols

6.1 Support protocol for yeast (e.g., *Saccharomyces cerevisiae*)

Optimal DNA yields from yeast samples can be obtained by following the standard protocol using NucleoSpin® Bead Tube Type C (see ordering information on section 7.2) instead of NucleoSpin® Bead Tube Type B provided with the NucleoSpin® Microbial DNA kit.

The agitation is recommended at a Retsch® Schwingmühle MM300: 12 min at 30 Hz. For other disruption devices, please check section 2.4. Please note that the position of the tube within the machine is important for optimal performance, please consult instruction manual of the machine.

If bead carryover is observed in the eluate, transfer the eluate into a new 1.5 mL nuclease-free tube carefully avoid disturbing the pellet.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Incomplete lysis* • Adjust lysis conditions (bead tube type, agitation device, duration, or frequency). *Reagents not applied properly* • Prepare Buffer B5 according to instructions (section 3). *Suboptimal elution of DNA from the column* • For certain sample types, preheat Buffer BE to 70 °C before elution. Apply Buffer BE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically, if elution is done with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer BE (pH 8.5). • Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL Buffer BE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation.
Poor DNA quality	*High A_{260}/A_{280} ratio* • Ratios > 1.9 can be caused by RNA contamination. Usually, such RNA contamination do not interfere with downstream application. Depending on sample type, amount, and disruption procedure, preparations might contain small amounts of RNA. If it is necessary to reduce RNA contamination to the lowest possible level, incubate the lysate after the disruption step for 5 min at 70 °C in order to inactivate the Proteinase K. After cooling to room temperature add 20 µL RNase A (20 mg/mL) and incubate 5 min. Continue with the application of the lysate onto the column. *Reagents not applied properly* • Prepare Buffer B5 according to instructions (see section 3).
Clogged columns	*Too much sample material used* • Make sure to centrifuge the lysate after cell disruption in order to sediment beads and cell debris. Only transfer cleared supernatant onto the column.

Suboptimal performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol or salt* • Make sure to centrifuge ≥ 1 min at 11,000 x *g* in order to remove all of ethanolic Buffer B5 before eluting the DNA. If, for any reason, the level of Buffer B5 has reached the column outlet after drying, repeat the centrifugation. • Do not chill Buffer B5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer B5 to room temperature before use. *Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE.

7.2 Ordering information

Product	REF	Pack of
NucleoSpin® Microbial DNA	740235.10 / .50	10 / 50 preps
NucleoSpin® Soil	740780.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® Bead Tube Type A (0.6–0.8 mm ceramic beads) (recommended for soil and sediments)	740786.50	50 pieces
NucleoSpin® Bead Tube Type B (40–400 µm glass beads) (recommended for bacteria)	740812.50	50 pieces
NucleoSpin® Bead Tube Type C (1–3 mm corundum) (recommended for yeasts)	740813.50	50 pieces
Buffer BE	740306.100	125 mL
Buffer B5 Concentrate (for 125 mL Buffer B5)	740921	25 mL
Buffer BW	740922	100 mL
Liquid Proteinase K	740396	5 mL
RNase A	740505.50 740505	50 mg
Collection Tubes (2 mL)	740600	1000

7.3 Product use restriction/warranty

NucleoSpin® Microbial DNA kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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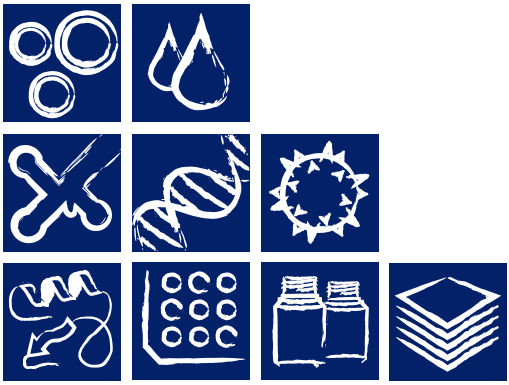

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