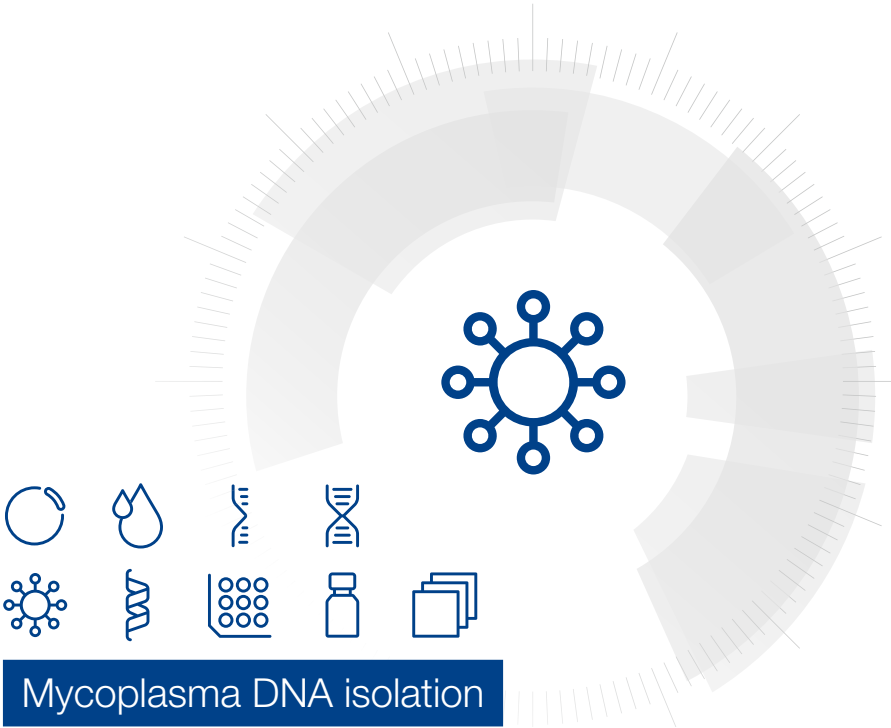


MACHEREY-NAGEL

User manual



Mycoplasma DNA isolation

■ NucleoSpin® Mycoplasma DNA

March 2024 / Rev. 02

Contact MN

Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valenciener 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

Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Reagents, consumables, and equipment to be supplied by user	5
1.3	About this user manual	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	General information on mycoplasma	7
2.4	Remarks regarding sample quality and preparation	7
2.5	Remarks regarding elution	7
2.6	Remarks regarding process hygiene	8
2.7	Remarks regarding quality control	8
3	Storage conditions and preparation of working solutions	9
4	Safety instructions	10
4.1	Disposal	10
5	Protocols	11
5.1	DNA extraction	11
6	Appendix	14
6.1	Troubleshooting	14
6.2	Ordering information	14
6.3	Validation data	15
6.4	Product use restriction/warranty	20

1 Components

1.1 Kit contents

NucleoSpin® Mycoplasma DNA	
REF	50 preps 740860.50
Lysis Buffer SML	25 mL
Wash Buffer SMW1	30 mL
Wash Buffer SMW2 (Concentrate)*	12 mL
RNase-free H ₂ O	13 mL
Carrier RNA (lyophilized)	2 × 300 µg
Liquid Proteinase K	600 µL
NucleoSpin® Mycoplasma DNA Columns (light red rings, plus Collection Tubes)	50
Collection Tubes (2 mL)	150
Collection Tubes (1.5 mL) for lysis and elution	100
Leaflet	1

* For preparation of working solutions and storage conditions see section 3.

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

Reagents

- 96–100 % ethanol (to prepare Wash Buffer SMW2 and to adjust DNA binding conditions); non-denaturated ethanol is recommended

Consumables

- Disposable pipette tips (aerosol barrier pipette tips are recommended to avoid cross-contamination)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating block for 56 °C incubation
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- Sterile working area (e.g. laminar airflow cabinet)

1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual if using the **NucleoSpin® Mycoplasma DNA** 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 referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Mycoplasma DNA** method, mycoplasmas are lysed quickly and efficiently by Lysis Buffer SML which is a highly concentrated solution of chaotropic ions. Lysis buffer and ethanol create appropriate binding conditions to bind nucleic acids to the NucleoSpin® Mycoplasma DNA Columns. Carrier RNA improves binding and recovery of low-concentrated nucleic acids. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in simple wash steps with alcoholic buffers SMW1 and SMW2. The nucleic acids are eluted in water and are ready-for-use in subsequent reactions.

2.2 Kit specifications

NucleoSpin® Mycoplasma DNA kit is designed for the rapid preparation of DNA from bioprocess samples for mycoplasma DNA contamination testing.

As sample material cell culture suspension, cells (pelleted), cell culture media, and cell culture supernatant can be used.

- No cross-contamination due to closed systems.
- The NucleoSpin® Mycoplasma DNA kit is suited to process 200 µL liquid sample or pelleted material.
- The NucleoSpin® Mycoplasma DNA Column allows a small elution volume (34 µL) for highly concentrated DNA.
- The prepared nucleic acids are suitable for qPCR Mycoplasma DNA contamination testing.
- The NucleoSpin® Mycoplasma DNA kit is suitable for mycoplasma testing according to EP 2.6.7 and JP G3.
- As qPCR kit, the TaKaRa Mycoplasma qPCR Detection Kit (Takara Bio Inc., Cat. #RR277A) is recommended. Other Mycoplasma PCR kit might be suitable.
- Every lot of NucleoSpin® Mycoplasma DNA kit is tested with a mycoplasma qPCR detection kit for non-detectable mycoplasma DNA contamination within the NucleoSpin® kit. Lot specific CoAs are available on the internet at www.mn-net.com.
- Carrier RNA (poly(-A) RNA: poly(A) potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance.
- Liquid Proteinase K is included to facilitate adequate lysis of proteins in the samples.
- For research use only.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Mycoplasma DNA
Technology	Silica-membrane technology
Format	Mini spin columns
Sample material	2 mL cell culture suspension, up to approx. 5×10^6 cells (pelleted), cell culture media, cell culture supernatant (2 mL).
Fragment size	approx. 100 bp–50 kb
Elution volume	34 μ L
Preparation time	50 min/6 preps

2.3 General information on mycoplasma

Mycoplasma is a group of bacteria that differ significantly from conventional bacteria due to their unique characteristics. Unlike most bacteria, mycoplasma lack a cell wall, making them the smallest self-replicating organisms. These microorganisms are ubiquitous in nature and can be found in various environments, including soil, water, saliva, and the respiratory and urogenital tracts of animals and humans.

Their ability to thrive in diverse conditions raises concerns about their impact on eukaryotic cells, particularly in the context of biopharmaceutical production processes. Mycoplasma contamination poses a substantial risk to cell cultures used in the production of biopharmaceuticals, as these bacteria can adversely affect the quality and safety of the final product.

Mycoplasma contamination can influence eukaryotic cells in multiple ways. They are known to disrupt cellular functions, compromise protein expression, and induce changes in cell morphology. As a result, the presence of mycoplasma can significantly impact the integrity and reliability of cell-based assays and experiments, leading to unreliable research outcomes and potentially endangering the safety and efficacy of biopharmaceutical products.

2.4 Remarks regarding sample quality and preparation

Cell culture supernatant, cells (pelleted), and cell culture media can be processed with NucleoSpin® Mycoplasma DNA kit. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample before loading onto the NucleoSpin® column. Therefore, check all samples for precipitates.

2.5 Remarks regarding elution

Standard elution volume is 34 μ L. This volume is small and sufficient to serve as template for e.g. the TaKaRa Mycoplasma qPCR Detection Kit (REF RR277A), which requires 13 + 13 + 1 μ L eluate for subsequent PCR analysis.

Elution volume can be reduced to 30 μ L when less eluate is sufficient. However, a column dead volume of approximately 5 μ L needs to be considered!

Elution volume can be increased up to 100 μ L, if higher elution volume is required.

2.6 Remarks regarding process hygiene

Mycoplasmas are widespread and commonly found in e.g. saliva and dust. Therefore, it is very important to keep a high level of hygiene in order to prevent contamination of samples, kit components and PCR reagents.

The following points are recommended:

- Wear lab coat, gloves – change gloves frequently.
- Never continue using gloves if they touch e.g. nose, glasses or unclean surfaces.
- Wear mouth-nose protection.
- Avoid sneezing, coughing, talking.
- Work in a clean-bench; prepare clean-bench in advance (UV, prerun).
- Clean/ decontaminate the centrifugal rotor and its lid before use.
- Open bottles and columns only in a clean-bench; open them as long as necessary and as short as possible.
- Use fresh and sterile pipette tips.
- Never use a pipette tip, that has contacted surfaces like e.g. work bench surface.
- Work prudent and concentrated - avoid hectic and quick movements.
- Follow general recommendations for sterile working.

2.7 Remarks regarding quality control

In accordance with MACHEREY-NAGEL's Quality Management System, each component of **NucleoSpin® Mycoplasma DNA** kits is tested against predetermined specifications to ensure consistent product quality.

Every lot of NucleoSpin® Mycoplasma DNA kit is tested with a mycoplasma qPCR detection kit for non-detectable mycoplasma DNA contamination. Lot specific CoAs are available on the internet at www.mn-net.com.

3 Storage conditions and preparation of working solutions

Attention: Buffers SML and SMW1 contain guanidine salts! Wear gloves and goggles!

- Check all components for damages after receiving the kit. If kit contents like buffer bottles or blisters packages are damaged, contact MACHEREY-NAGEL. Do not use damaged kit components.
- Upon arrival, the **NucleoSpin® Mycoplasma DNA** kit should be stored at room temperature (15–25 °C). It is NOT required to open the kit on delivery and remove individual components for separate storage.
- After first time use, it is recommended to store Liquid Proteinase K at 4 °C or -20 °C.
- Use RNase-free equipment.

Before starting any **NucleoSpin® Mycoplasma DNA** preparation prepare the following:

- **Carrier RNA** (300 µg) is delivered in lyophilized form. Dissolve Carrier RNA in RNase-free water to obtain a stock solution (1 µg/µL). Store Carrier RNA stock solution at -20 °C. Due to the production procedure and the small amount of Carrier RNA contained in the vial, the Carrier RNA may hardly be visible in the vial.
- **Wash Buffer SMW2**: Add the indicated volume (see on the bottle or table below) of ethanol (96–100 %; non-denatured ethanol is recommended) to **Wash Buffer SMW2 Concentrate**. Mark the label of the bottle to indicate that the ethanol is added. Store **Wash Buffer SMW2** at room temperature.
- **Liquid Proteinase K** is ready to use. After first time use, store liquid Proteinase K at 4 °C or -20 °C.

NucleoSpin® Mycoplasma DNA	
REF	50 preps 740860.50
Wash Buffer SMW2 (Concentrate)	12 mL Add 48 mL ethanol to each bottle
Carrier RNA	300 µg Add 300 µL RNase-free H ₂ O

4 Safety instructions

When working with the **NucleoSpin® Mycoplasma DNA** kit 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).



Caution: Guanidine hydrochloride in buffer SML and buffer SMW1, can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® Mycoplasma DNA** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

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 DNA extraction

Before starting the preparation:

- Check if Wash Buffer SMW2 was prepared according to section 3.
 - Check if Carrier RNA is dissolved in water (stock solution).
 - The complete procedure should be performed at room temperature.
 - Preheat RNase-free H₂O (for elution) to 70 °C.
-

0 Provide sample

Cells

Centrifuge 2 mL of cell suspension (approximately 2×10^6 cells) at 15,000 rpm for 5 min (1.5 mL Collection Tube, provided).

Remove and discard 1,810 µL of supernatant, leave 190 µL in the tube.

Cell supernatant, cell media

Provide 190 µL in a tube (1.5 mL Collection Tube, provided)

Add **10 µL of Spike-in Control DNA** (not included in the NucleoSpin® kit; part of TaKaRa Mycoplasma qPCR Detection Kit). Volume of Spike-in Control DNA might vary for other PCR kits!

1 Lyse sample

Add **5 µL Liquid Proteinase K** into the Tube.

Note: Proteinase K may be pipetted into the inside of the lid or onto the side of the inner tube wall. Visually inspect, that the Liquid Proteinase K has been actually deposited into the tube or lid!

Mix moderately.

Add **200 µL Lysis Buffer SML** to the tube.

Mix the tube content moderately by vortexing (10–15 s).

If necessary, briefly centrifuge the Collection Tube (**~ 1 s at ~ 2,000 × g**) to remove drops from the lid (short spin only).

Add **5.6 µL Carrier RNA** stock solution (1 µg/µL) to the tube.

Mix the tube content by vortexing or pipetting up and down.

Incubate for **3 min at room temperature**.

If necessary, briefly centrifuge the Collection Tube (**~ 1 s at ~ 2,000 × g**) to remove drops from the lid (short spin only).



**5 µL
Proteinase K**

**+200 µL
sample**

+200 µL SML

Mix

**~ 1 s,
~ 2,000 × g**



**+5.6 µL Carrier
RNA**



Mix

RT, 3 min

**~ 1 s,
~ 2,000 × g**

**2 Adjust binding conditions**

Add **200 µL ethanol** (96–100 %) to the tube and mix by vortexing (10–15 s).

Incubate for **5 min at room temperature**.

If necessary, briefly centrifuge the Collection Tube (**~ 1 s at ~ 2,000 × g**) to remove drops from the lid (short spin only).

Do not centrifuge at a higher g-force in this step!



+200 µL EtOH

RT, 5 min

**~ 1 s,
~ 2,000 × g**

**3 Bind DNA**

Load the lysate (approx. 610 µL) onto a NucleoSpin® Mycoplasma DNA Column and centrifuge **3 min at 4,000 × g**.

If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-forces (15,000–20,800 × g for 1 min). In case the lysate still does not pass the membrane completely, discard the sample and repeat the isolation with new sample material.

Place the NucleoSpin® Mycoplasma DNA Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.



Load sample

**3 min,
4,000 × g**

4 Wash and dry silica membrane**1st wash**

Add **400 µL Wash Buffer SMW1** to the NucleoSpin® Mycoplasma DNA Column.

Centrifuge **30 s** at **11,000 × g**.

Place the NucleoSpin® Mycoplasma DNA Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.



+400 µL SMW1

30 s,
11,000 × g

2nd wash

Add **400 µL Wash Buffer SMW2** to the NucleoSpin® Mycoplasma DNA Column.

Centrifuge **30 s** at **11,000 × g**.

Place the NucleoSpin® Mycoplasma DNA Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.



+400 µL SMW2

30 s,
11,000 × g

Note: Make sure that residual buffer from the previous step is washed away with Buffer SMW2 especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim, flush it with Buffer SMW2

3rd wash

Add **200 µL Wash Buffer SMW2** to the NucleoSpin® Mycoplasma DNA Column.

Centrifuge **for 5 min** at **20,000 × g** (or full speed).

Place the NucleoSpin® Mycoplasma DNA Column in a clean Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.

Incubate the assembly for **5 min** at **56 °C** with open column lid.



+200 µL SMW2

5 min,
20,000 × g

56 °C, 5 min**5 Elute DNA**

Add **34 µL RNase-free H₂O** (pre-heated to 70 °C) onto the column.

Incubate for **3 min** at **room temperature**.

Centrifuge **3 min** at **20,000 × g** to elute nucleic acid from the column.

Keep eluted DNA on ice or freeze for storage.

Note: For alternative elution volumes see section 2.5.



+34 µL
RNase-free
H₂O (70 °C)

RT, 3 min

3 min,
20,000 × g

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Problems with subsequent detection	<p>Reduced sensitivity</p> <ul style="list-style-type: none"> Change the volume of eluate added to the PCR/RT-PCR.
	<p>Ethanol carry-over</p> <ul style="list-style-type: none"> Prolong centrifugation steps in order to remove Buffer SMW2 completely.
	<p>Carrier RNA interference with detection method</p> <ul style="list-style-type: none"> Check if Carrier RNA interferes the detection method. Some detection methods tolerate only limited amounts of carrier RNA.
General problems	<p>Clogged membrane</p> <ul style="list-style-type: none"> Use less cell material.
	<p>Mycoplasma contamination in negative controls.</p> <ul style="list-style-type: none"> Make sure to follow rigorous hygiene standards for DNA extraction as well as PCR setup (see section 2.6)!

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® Mycoplasma DNA	740860.50	50
Liquid Proteinase K	740396	5 mL
MN Bead Tubes Type A (DNA and nuclease free)	740786.50	50

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

6.3 Validation data

The following data are provided to support users of the NucleoSpin® Mycoplasma DNA kit to comply with the regulation of the Japanese Pharmacopoeia (G3 section 14) concerning Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological / Biological Products.

The user should confirm the provided validation results by his own facilities in order to fully comply with the above mentioned regulation!

The NucleoSpin® Mycoplasma DNA kit in conjunction with the Takara Mycoplasma qPCR Detection Kit is targeting a highly conserved region within the mycoplasma genome to detect all species included in the Japanese Pharmacopoeia Chapter G3 (JP G3) and many more.

The kit was validated according to JP G3 section 14 with respect to detection limit for all listed mycoplasma species, specificity and robustness. However, please note that these validation data are provided for information purposes only. The JP G3 – 14 clearly states:

The user should confirm the manufacturer's validation results by own facilities. ... Where commercial kits are used for a part or all of the analytical procedure, documented full validation data already covered by the kit manufacturer can replace validation data by the user, and a full validation by the user is unnecessary. Nevertheless, the performance of the kit with respect to its intended use and user's test system should be demonstrated by the user (e.g. specificity, detection limit).

Please feel free to contact us if you need further assistance.

Validation data presented was obtained using the NucleoSpin® Virus kit, which is a technical equivalent to the NucleoSpin® Mycoplasma DNA kit. The NucleoSpin® Mycoplasma DNA kit is produced following higher hygiene standards and every lot is tested for nondetectability of mycoplasma contamination. Please feel free to contact us if you need a detailed technical comparison of the two kits. Data kindly provided by Takara Bio Inc..

Specificity part 1

Method

Detection by real-time PCR was performed using 7 mycoplasma genomic DNAs available commercially. Approximately 10 µg of DNA was used as a template and duplicate detection (N= 2) was performed for each sample.

Results

Table 2 shows the mycoplasma species used and the Ct values obtained from real-time PCR. Excellent detection performance was observed with all 7 species.

Table 2. Mycoplasma species

No.	Species	ATCC No.	Template	Ct1	Ct2	Average
1	<i>Mycoplasma arthritidis</i>	19611D	10 µg	27.4	27.4	27.4
2	<i>Mycoplasma bovis</i>	25523D	10 µg	24.4	24.5	24.4
3	<i>Mycoplasma hominis</i>	23114D	10 µg	23.2	23.2	23.2
4	<i>Mycoplasma hyopneumoniae</i>	25934D	10 µg	24.0	24.0	24.0
5	<i>Mycoplasma pirum</i>	25960D	10 µg	26.4	26.4	26.4
6	<i>Mycoplasma synoviae</i>	qCRM-25204D	10 ⁴ copies	23.5	23.4	23.4
7	<i>Spiroplasma citri</i>	27556D-5	10 µg	25.9	26.0	25.9

Specificity part 2

Method

Detection by real-time PCR was performed using 13 bacterial genomic DNAs available commercially. Approximately 100 µg of DNA was used as a template and duplicate detection (N= 2) was performed for each sample.

Result

Table 3 shows the bacterial species used and the Ct values obtained from realtime PCR. Cross-reactivity was not detected with any bacterial genomic DNA.

Table 3. Bacteria other than Mycoplasma

No.	Species	NBRC No.	Template	Ct1	Ct2
1	<i>Bacillus subtilis</i>	13719G	100 pg	–	–
2	<i>Brevibacillus brevis</i>	100599G	100 pg	–	–
3	<i>Clostridium acetobutylicum</i>	13948G	100 pg	–	–
4	<i>Clostridium kluyveri</i>	12016G	100 pg	–	–
5	<i>Escherichia coli</i>	12713G	100 pg	–	–
6	<i>Klebsiella pneumoniae</i>	14940G	100 pg	–	–
7	<i>Pseudomonas aeruginosa</i>	106052G	100 pg	–	–
8	<i>Salmonella enterica subsp. enterica</i>	13245G	100 pg	–	–
9	<i>Staphylococcus aureus</i>	100910G	100 pg	–	–
10	<i>Streptococcus mutans</i>	13955G	100 pg	–	–
11	<i>Streptomyces avermitilis</i>	14893G	100 pg	–	–
12	<i>Rhodococcus erythropolis</i>	100887G	100 pg	–	–
13	<i>Tetragenococcus halophilus</i>	12172G	100 pg	–	–

Sensitivity

Method

The cut-off value for obtaining a 95 % positive result using this kit was determined using mycoplasma standards for 7 different species (Table 3) from ATCC. 1, 10, and 100 cfu/ml of the mycoplasma standards were added to 10^6 cells/ml of CHO cells. (0.1, 1, and 10 ccu/ml for *M. pneumoniae*). DNA from CHO cells containing added mycoplasma was extracted and analyzed using this kit. The mycoplasma standards were diluted stepwise in 4 sets of dilutions. DNA extraction and real-time PCR were repeated 6 times for each 10 cfu/ml mycoplasma addition, providing results for a total of 24 data points. For the 1 cfu/ml and 100 cfu/ml mycoplasma additions, DNA extraction and real-time PCR were repeated twice, providing results for a total of 8 data points.

Table 4. Mycoplasma standards for different species

Species	ATCC No.	Lot No.	Post-preservation titer	Genome copy (GC)
<i>Mycoplasma arginini</i>	23838-TTR	60224014	3.70×10^9 cfu/ml	8.93×10^9 GC/ml
<i>Mycoplasma fermentans</i>	19989-TTR	60316337	1.00×10^9 cfu/ml	8.61×10^9 GC/ml
<i>Mycoplasma salivarium</i>	23064-TTR	60171952	1.67×10^9 cfu/ml	3.80×10^9 GC/ml
<i>Mycoplasma orale</i>	23714-TTR	61060921	3.08×10^8 cfu/ml	1.54×10^9 GC/ml
<i>Mycoplasma hyorhinis</i>	17981-TTR	63478133	8.77×10^8 cfu/ml	1.23×10^9 GC/ml
<i>Acholeplasma laidlawii</i>	23206-TTR	60171953	7.10×10^8 cfu/ml	5.86×10^9 GC/ml
<i>Mycoplasma pneumoniae</i>	15531-TTR	60171955	1.00×10^8 ccu/ml	5.82×10^9 GC/ml

Result

The average and standard deviation of Ct values for each test are presented in Table 5. The number and percentage of results judged as positive * are shown in Table 5. The cut-off values showing a 95 % positive result are 10 cfu/ml or less for all 7 species of mycoplasma (1 ccu/ml for *M. pneumoniae*).

* Test results showing Ct values of one or two reactions in duplicate are judged as "Positive".

Table 5. Ct values

Species	Average			SD		
	100 cfu/ml	10 cfu/ml	1 cfu/ml	100 cfu/ml	10 cfu/ml	1 cfu/ml
<i>M. arginini</i>	33.0	36.2	39.7	0.356	0.995	2.003
<i>M. fermentans</i>	31.9	35.5	38.8	0.385	0.828	0.952
<i>M. salivarium</i>	33.3	37.0	39.2	0.532	0.857	1.853
<i>M. orale</i>	33.8	38.1	39.1	0.293	1.336	0.650
<i>M. hyorhinis</i>	31.3	35.4	39.7	0.402	0.550	2.470
<i>A. laidlawii</i>	30.6	35.2	39.2	0.512	1.908	1.585
	10 ccu/ml	1 ccu/ml	0.1ccu/ml	10 ccu/ml	1 ccu/ml	0.1 ccu/ml
<i>M. pneumoniae</i>	33.2	36.5	40.0	0.397	1.132	1.898

Table 6. Number and percentage of positive test results

Species	Average			SD		
	100 cfu/ml	10 cfu/ml	1 cfu/ml	100 cfu/ml	10 cfu/ml	1 cfu/ml
<i>M. arginini</i>	8/8	24/24	6/8	100.0	100.0	75.0
<i>M. fermentans</i>	8/8	24/24	5/8	100.0	100.0	62.5
<i>M. salivarium</i>	8/8	24/24	6/8	100.0	100.0	75.0
<i>M. orale</i>	8/8	24/24	5/8	100.0	100.0	62.5
<i>M. hyorhinis</i>	8/8	24/24	8/8	100.0	100.0	100.0
<i>A. laidlawii</i>	8/8	24/24	8/8	100.0	100.0	100.0
	10 ccu/ml	1 ccu/ml	0.1 ccu/ml	10 ccu/ml	1 ccu/ml	0.1 ccu/ml
<i>M. pneumoniae</i>	8/8	24/24	3/8	100.0	100.0	37.5

Robustness

Method

Three independent kit lots of NucleoSpin® Mycoplasma DNA were produced. Two operators, each in another lab facility, performed 8 mock DNA isolations with every of the three kit lots using ultra clean water as sample material. According to the user manual, all samples were spiked with a spike-in DNA control. Eluates were analyzed with the Takara Mycoplasma qPCR Detection Kit according to the instruction manual.

Results

The Ct values obtained in the qPCR analysis for the spike-in DNA control show within run variation, between run variation, lot to lot variation, and operator to operator variation. Standard deviation over all 72 data points is 0.46 cycles indicating a high robustness of the system

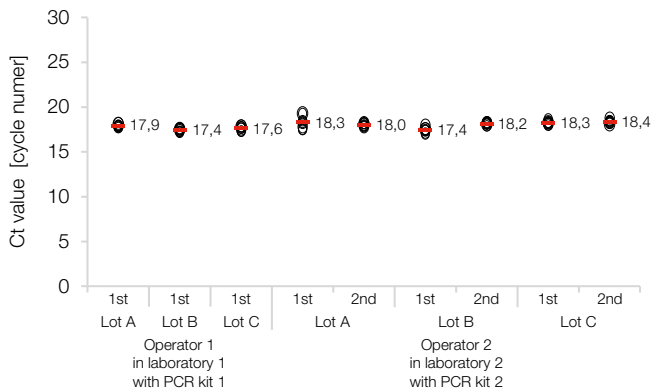


Figure 7: Robustness of the NucleoSpin® Mycoplasma DNA kit in conjunction with the Takara Mycoplasma qPCR Detection Kit. 1st denotes a first run, 2nd denotes a second run. Black circles indicated individual data points. Red bars indicate the average of eight data points.

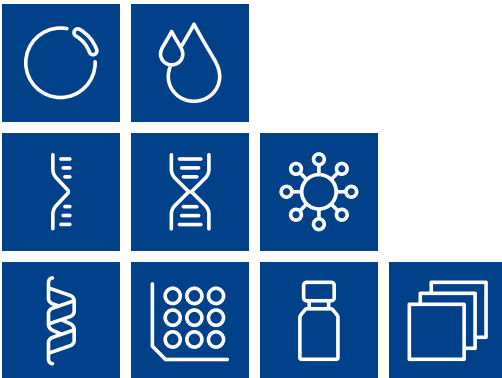
6.4 Product use restriction/warranty

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.

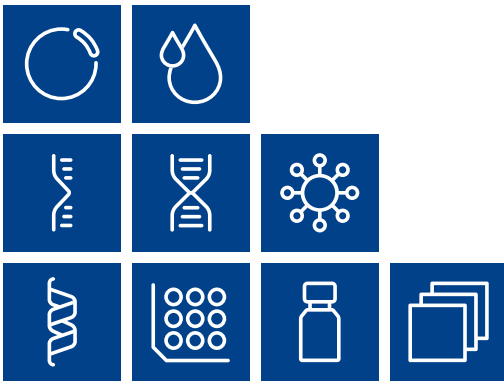
This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No 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 customer 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.



Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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

