For customers in the European Economic Area: Contains SVHC: octyl/nonylphenol ethoxylates. For use in analytical activities such as monitoring and quality control and under controlled conditions only– acc. to Art. 56 (3) and 3 no. 23 REACH Regulation.

MycoTOOL Mycoplasma Real-Time PCR Kit

Version 10

Content version: August 2020

For the testing of cell culture samples for the absence of mycoplasmas

REF 06 495 605 001 1 kit

160 PCR amplifications with
50 µl final reaction volume

Store the kit at –15 to –25°C.

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Table of Contents

1. What this Product Does

Number of Tests

The MycoTOOL Mycoplasma Real-Time PCR Kit is designed to test at least 10 cell culture samples for the absence of Mycoplasma. The kit contains sufficient reagents to run 160 PCRs each with a 50 µl reaction volume.

Kit Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	Recovery Control	Plasmid	1 vial, 400 µl
2	red	PCR Master, 2× conc.	Contains all reagents needed for running real-time DNA detection assays, except primer and template	5 vials, 1 ml each
3	orange	UNG	Enzyme that digests dUTP-containing DNA	1 vial, 180 µl
4	turquoise	PCR Enhancer	PCR Additive	1 vial, 180 µl
5	green	Detection Mix, 25× conc.	Primers and FAM-labeled detection probe	1 vial, 220 µl
6	yellow	Detection Mix Recovery Control, 25× conc.	Primers and LightCycler® Yellow 555-labeled detection probe	1 vial, 140 µl
7	purple	Positive Control	Plasmid	1 vial, 800 µl
8	white	Water, PCR grade		2 vials, 1 ml each

Storage and Stability

The product is shipped on dry ice.

When stored at -15 to -25°C, the kit is stable until the expiration date printed on the label.

⚠ After opening the kit, store Vials 5 and 6 protected from light.

⚠ Avoid repeated freezing and thawing of Vial 2. After thawing, store Vial 2 for up to 4 weeks at +2 to +8°C.

Assay Time

Hands-on time PCR setup: approximately 1 hour.

Total time-to-result (without sample preparation): **approximately 4 hours**

Additional Equipment and Reagents Required

- Pipettes
- Nuclease-free, DNA-free, aerosol-resistant pipette tips
- Nuclease-free, DNA-free vials
- Alcohol wipes
- Biosafety cabinet class II
- Thermomixer (for 2 ml tubes)
- Benchtop centrifuge (for 2 ml tubes)
- Vortex mixer

For the nucleic acid isolation

To prepare the sample material for the analysis, choose

- an automated procedure using the MagNA Pure 96 Instrument* with the MagNA Pure 96 DNA and Viral NA Large Volume Kit*,
- or a manual procedure using the QC Sample Preparation Kit*.

For the PCR workflow

- Laminar flow hood
- Real-time PCR Instrument carrying at least two detection channels (FAM, VIC/ HEX/ Yellow555) with accessories and disposables. We recommend the LightCycler® 480 Instrument II* (96-well version).
- Multiwell plates: LightCycler® 480 Multiwell Plate 96, white*.
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.

Applications

The MycoTOOL Mycoplasma Real-Time PCR Kit is an *in vitro* nucleic acid amplification test optimized for the detection of Mycoplasma in CHO cell culture, according to the Nucleic Acid Amplification Tested (NAT) guidelines for Mycoplasma described in Chapter 2.6.7 of the European Pharmacopoeia, with respect to specificity, sensitivity/detection limit, and robustness.

The performance criteria of this kit when testing other cell lines, such as NS0 and SP2, need to be established by the user.

The kit was developed according to the cfu specifications provided by suppliers of test materials (e.g., European Directorate for the Quality of Medicines & Health Care).

Specificity and Sensitivity

The specificity of the MycoTOOL Mycoplasma Real-Time PCR Kit assay is tested for the absence of interference with *Streptococcus bovis* (ATCC 9809), *Lactobacillus acidophilus* (ATCC 4356), and *Clostridium sporogenes* (ATCC 11437), according to the European Pharmacopoeia.

The kit was developed to meet the sensitivity requirements indicated in the European Pharmacopoeia, Chapter 2.6.7.

2. How to Use this Product

2.1 Before You Begin

Safety Information

For customers in the European Economic Area: Contains SVHC: octyl/nonyl-phenol ethoxylates. For use in analytical activities such as monitoring and qual-ity control and under controlled conditions only– acc. to Art. 56 (3) and 3 no. 23 REACH Regulation.

Precautions

This product contains a substance on REACH Annex XIV (substance of very high concern due to endocrine disrupting properties for the environment) at or above 0.1% w/w. This product may only be used under the exemption from authorization for scientific research and development (including analytical activities, quality control, and In-Vitro Diagnostics) under controlled conditions. Only trained and authorized personnel are allowed to handle the substance.

To avoid contamination, perform the workflow setup under DNA-free conditions. This includes:

- Prepare and pipette all solutions with nuclease-free, DNA-free equipment and consumables.
- UV-treat the laminar flow hood prior to pipetting.
- Use sterile single-use gloves and freshly laundered laboratory coats.
- Close vials immediately after pipetting.
- Spatial segregation of the sequential workflow steps.

Rooms	Workflow Step
Sample preparation room	Extraction and purification of test samples, including preparation of recovery control sample.
Master mix preparation room	Master mix preparation and pipetting of PCR negative control to the NTC wells.
PCR room for setup and amplification run	Dilution and pipetting of samples and PCR Positive Control to the positive control wells. Running the LightCycler® 480 Instrument II.

Waste Handling Product: The unused or used product should not be allowed to enter drains, waterways, or the soil. Do not contaminate ponds, waterways, or ditches with chemicals or used containers. Collect the used and unused product separately and send it to a licensed waste management company for disposal. Contaminated packaging: Empty remaining contents. Dispose of as unused product. Empty containers are considered as packaging waste and should be taken to an approved waste handling site for disposal. Do not reuse empty containers.

2.2 Sample Preparation

In combination with this kit, there are two options for sample preparation, manual or automated. For both purification methods, the same sensitivity is achieved using 20 µl eluate in a 50 µl PCR.

⚠ Do not exceed a cell density of 5 x 10^6 cells/ml in the sample. Higher cell densities can lead to false-negative results due to inefficient sample preparation from overloaded purification matrix.

2.2.1 Automated Sample Preparation

For automated sample preparation, use the MagNA Pure 96 Instrument* with the MagNA Pure 96 DNA and Viral NA Large Volume Kit*. Please refer to the corresponding manuals to make best use of this instrument and its reagents.

In this instance, use the MagNA Pure 96 Instrument to purify up to 5 × 10^6 CHO cells/ml using the MagNA Pure 96 DNA and Viral NA Large Volume Kit with the MycoTOOL Mycoplasma Real-Time PCR Kit. Please note that the upper limit for other cell lines should be determined empirically by the user when using the MagNA Pure 96 System in conjunction with the MycoTOOL Mycoplasma Real-Time PCR Kit.

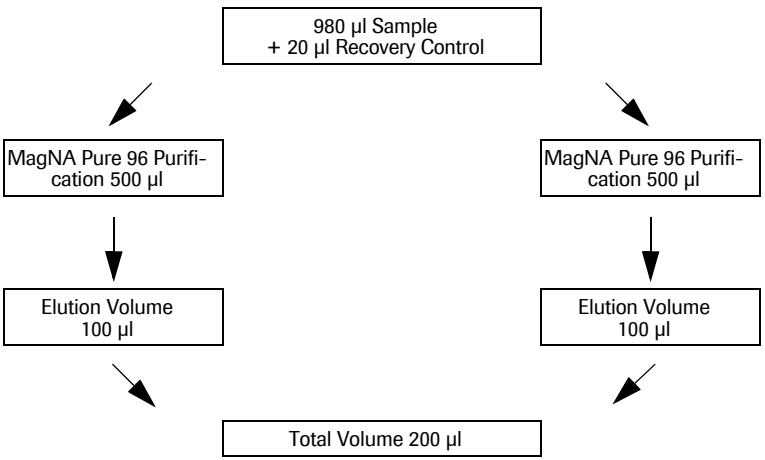


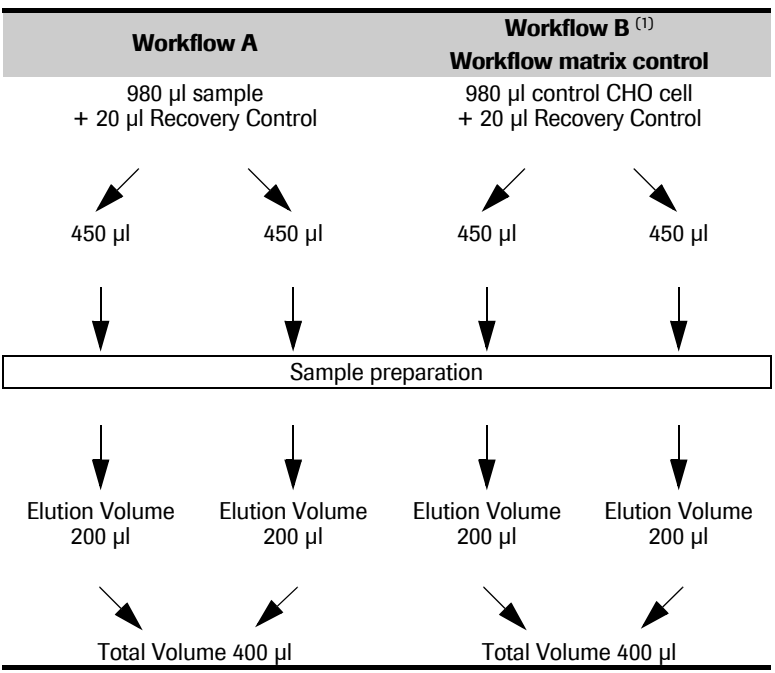
Fig. 1a: Experimental overview for the automatic sample preparation.

In addition to the guidelines provided in the respective MagNA Pure 96 Operator's Manual, do the following (Figure 1a):

❶	Prepare the MagNA Pure 96 Instrument according to the instructions in the Operator's Manual.
❷	Select the purification protocol "Viral NA Universal LV".
❸	Enter Sample Volume: 500 µl
❹	Enter Elution Volume: 100 µl
❺	Prepare 980 µl sample or workflow matrix control (1) and add 20 µl Recovery Control.
❻	Divide into 2 × 500 µl aliquots using a pipette.
❼	Start the MagNA Pure 96 DNA purification run.
❽	DNA is eluted two times using 100 µl volume. Pool these two eluates.

(1) Optional: Positive or negative control depending on customer requirements.

2.2.2 Manual Sample Preparation



(1) Optional: Positive or negative control depending on customer requirements.

Fig. 1b: Experimental overview of the manual sample preparation.

The following protocol describes the manual sample preparation using the QC Sample Preparation Kit*. The vials from the QC Sample Preparation Kit are marked with #.

❶	Equilibrate thermomixer to +56°C.
❷	▪ Prepare appropriate number of empty vials (Reagent Vials 8# from the QC Sample Preparation Kit) with 30 µl Proteinase K (Vial 1#) each. ▪ Label the Reaction Vials accordingly.
❸	Add 450 µl of sample or workflow matrix control to each Reaction Vial.
❹	Add 450 µl Lysis Buffer (Vial 2#) to each Reaction Vial.
❺	Close the Reaction Vials and vortex 3 times for 5 seconds.
❻	Incubate for 15 minutes at +56°C and 600 rpm in the thermomixer
❼	▪ Remove the Reaction Vials. ▪ Equilibrate the thermomixer to +80°C.
❽	▪ Add 630 µl Precipitation Reagent (Vial 3#) to each Reaction Vial. ▪ Close the Reaction Vials, invert 20 times, and vortex for 5 seconds.
❾	▪ Centrifuge for 3 minutes at 16,000 × *g*. ▪ Decant supernatant without removing pellet.
❿	Add 1 ml Washing Buffer (Vial 4#).
⓫	▪ Close the Reaction Vials and invert 5 times. ▪ Immediately centrifuge for 3 minutes at 16,000 × *g* and carefully remove all of the supernatant.
⓬	Briefly centrifuge for 3 seconds at 16,000 × *g* and carefully remove the residual supernatant.
⓭	Add 200 µl Dissolution Buffer (Vial 5#).
⓮	▪ Close the Reaction Vials and dissolve the pellet for 10 minutes at +80°C and 900 rpm in the thermomixer. Depending on the sample material, it may be beneficial to extend the incubation time up to 30 minutes.
⓯	Vortex until the pellet is completely dissolved. Pool these two eluates.
⓰	Transfer the Reaction Vials to the PCR room. Eluted DNA is stable for 3 days at -15 to -25°C.

2.3 Setting up the PCR Experiment

2.3.1 Plate Setup and Number of PCR Reactions

For each sample to be tested, prepare the following number of PCRs.

Run four replicates per PCR. Always run two PCR negative controls (NTC) with the Mycoplasma PCR and with the Recovery Control PCR.
To prepare a PCR negative control (NTC), replace the template DNA with Water, PCR Grade (Vial 8).
The figures below illustrate an example of a plate setup for only one sample (Figure 2a) and for more than one sample (Figure 2b), including all standards and controls.

	Sample PCR rxn[(1)]	Positive Control rxn	NTC (PCR negative control) rxn	Total rxn	Master Mix Preparation[(2)]	Master Mix [µl][(2)] 30 µl per reaction
Mycoplasma PCR	4	4	2	10	11	330
Recovery Control PCR	4	-	2	6	7	210

[(1)]rxn = reactions

[(2)]Calculated by adding one additional reaction to compensate for a slight loss of liquid during the pipetting steps.

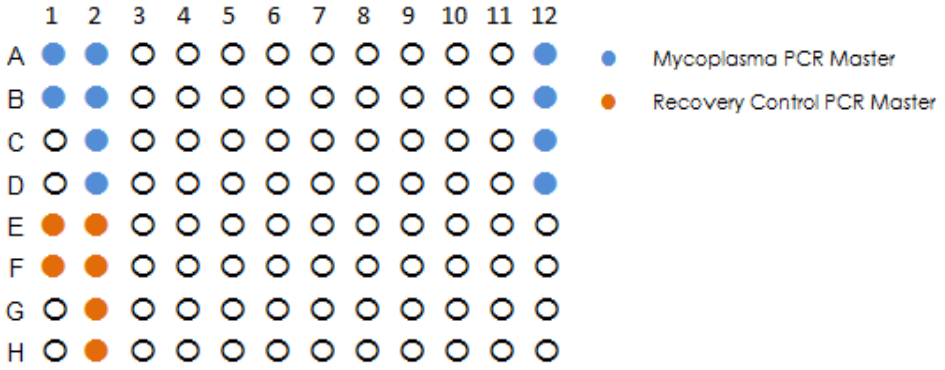


Fig. 2a: Plate configuration proposal for one sample per plate.

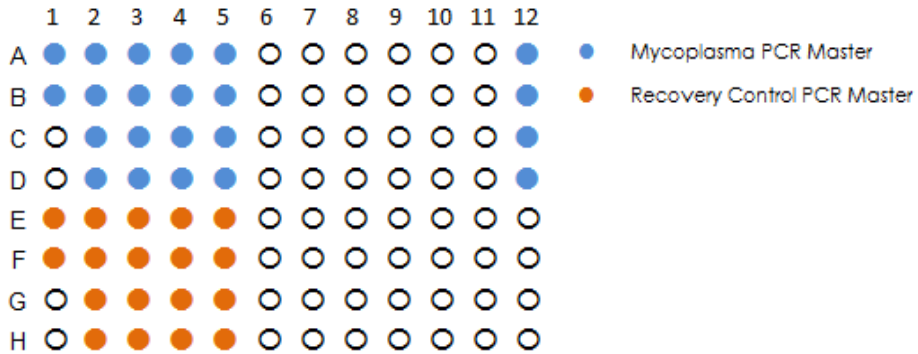


Fig. 2b: Plate configuration proposal for more than one sample per plate, for example, four samples per plate.

2.3.2 PCR with the LightCycler® 480 Instrument II

LightCycler® 480 Instrument II PCR Profile

For details on how to program the experimental protocol, see the current LightCycler® 480 Instrument II Operator's Manual.

Program the LightCycler® 480 Instrument II before preparing the reaction mixes. The temperature profile includes an initial incubation step at +40°C to allow UNG to digest dUTP containing DNA. The initial denaturation step will denature UNG and also activate the polymerase. Due to high genomic DNA background, a touchdown PCR protocol is required.

Program the PCR profile as indicated below and save it as a template file for reuse.

Setup	**Reaction Volume [µl]**	**Block Type**
	50	96
Detection Format	**Excitation Filter**	**Emission Filter**
Dual Color Hydrolysis Probe / UPL Probe		
FAM	465	510
VIC/Hex/Yellow 555	533	580
Programs		
Program Name	**Cycles**	Analysis Mode
UNG	1	None
Initial Denaturation	1	None
Pre-Amplification	2	None
Amplification	48	Quantification
Cooling	1	None

Temperature Targets						
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**	**Acquisitions [per/°C]**	**Sec Target [°C]**	**Step Size [°C]**
UNG						
40	None	00:10:00	4.4	–	–	–
Initial Denaturation						
95	None	00:10:00	4.4	–	–	–
Pre-Amplification						
95	None	00:00:15	4.4	–	–	–
70	None	00:00:15	2.2	–	–	–
72	None	00:00:20	4.4	–	–	–
Amplification						
95	None	00:00:15	4.4	–	–	–
69	None	00:00:15	2.2	–	60	0.5
72	Single	00:00:20	4.4	–	–	–
Cooling						
40	None	00:00:30	2.2	–	-	–

Preparation of the Master Mix

Since real-time PCR is an extremely sensitive method to detect traces of DNA, follow the appropriate guidelines for preparing PCR master mixes.

⚠ Keep Vials 5 and 6 away from light. Do not touch the surface of the LightCycler® 480 Multiwell Plate during handling.

❶	Perform laminar flow hood cleaning (using bleach, then ethanol or other disinfectant reagents) in the master mix room.
❷	Wipe pipetting tools with 10% bleach followed by 70% ethanol or other disinfectants. Wipe all other items with 70% ethanol before bringing into the hood.
❸	Place the reagents (see Step 6 below) in a laminar flow hood, and thaw at +15 to +25°C.
❹	Vortex and spin briefly before opening.
❺	Change tip after each pipetting step.
❻	For the plate setup (Figure 2a), prepare the two different master mixes according to the table below. Use nuclease-free, DNA-free vials.

Vial	Component	Mycoplasma PCR Master [µl] 1× rxn	Mycoplasma PCR Master [µl] 11× rxn[1]	Recovery Control PCR Master [µl] 1× rxn	Recovery Control PCR Master [µl] 7× rxn[1]
2	PCR Master, 2× conc.	25	275	25	175
3	UNG (2 U/µl)	1	11	1	7
4	PCR Enhancer	0.9	9.9	0.9	6.3
5	Detection Mix, 25× conc.	2	22		
6	Detection Mix Recovery Control, 25× conc.			2	14
8	Water, PCR Grade	1.1	12.1	1.1	7.7
	Total Volume	**30**	**330**	**30**	**210**

❼	Distribute 30 µl of the respective master mix into the respective well of a 96-well plate.
❽	Transfer the 96-well plate to PCR setup room.

[1]Calculated by adding one additional reaction to compensate for a slight loss of liquid during the pipetting steps (e.g., 10 reactions + 1 = 11 reactions).

Preparation of the PCR Plate and PCR Run

❶	Perform laminar flow hood cleaning (using bleach, then ethanol or other appropriate disinfectants) in the PCR setup room.
❷	Wipe the pipetting tools with 10% bleach followed by 70% ethanol or other disinfectants.
❸	Add 20 µl of the required sample material (sample, Positive Control, or NTC) to each well prefilled with master mix. Run replicates of each template as listed below.
❹	Load the prepared 96-well plate into the LightCycler® 480 Instrument II and start the run.

	Sample	Positive Control	NTC (PCR negative control)	Number of PCR Reaction
Mycoplasma PCRs	4 × 20 µl	4 × 20 µl	2 × 20 µl	10
Recovery Control PCRs	4 × 20 µl		2 × 20 µl	6

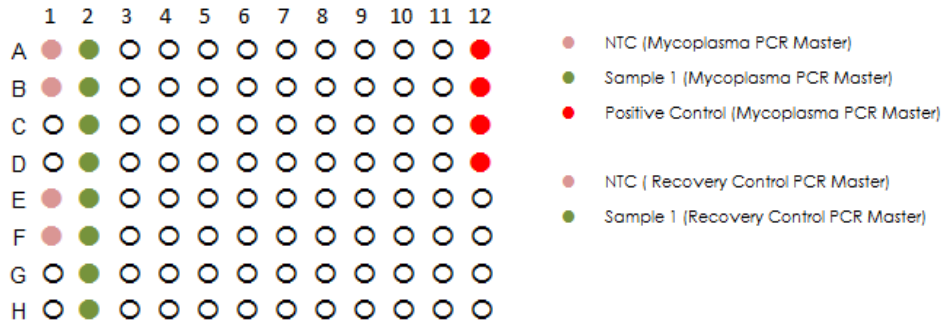


Fig. 3a: Plate configuration proposal, one sample per plate.

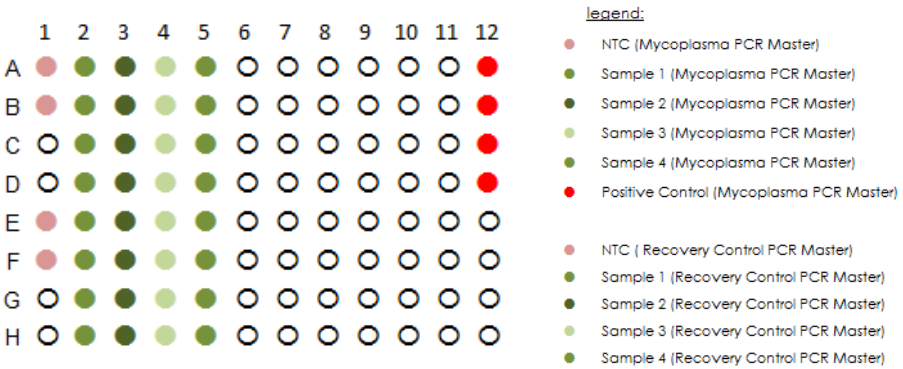


Fig. 3b: Plate configuration proposal for more than one sample, for example, four samples per plate.

Data Analysis

For the data analysis with the LightCycler® 480 Instrument II, Abs Quant/2nd Derivative Max for All Samples is recommended. Use the Filter Comb button to select the fluorescence channel to be analyzed. Channel FAM [465-510] for the Mycoplasma Master mix Probes and channel VIC/ HEX /Yellow555 [533-580] for the Recovery Control Master mix Probes. The use of subset is optional.

For more information, refer to the LightCycler® 480 Instrument II Operator's Manual.

3. Result Interpretation

3.1 Results with the LightCycler® 480 Instrument II

⚠ The validation of the workflow and the setting of the cut off value is under the responsability of the customer.

The following interpretation of the results is only an example.
In the analysis mode, the LightCycler® Instrument Software calls each well positive (red), negative (green), or uncertain (blue).
When the Software calls a well as uncertain, the respective sample must be retested.

⚠ Due to the Abs Quant/2nd Derivative Max method the latest Cp that the software can calculate is 43.0. Samples with Cp ≥ 43 have a higher uncertainty and should be evaluated carefully.

To indicate mycoplasma was detected in a sample, the results from Subset 1 (Mycoplasma PCR) and Subset 2 (Recovery Control PCR) are combined and interpreted in the following way:

A sample is negative when the following criteria are fulfilled:

Subset 1:

1. NTC: All (2/2) NTCs have to be negative
2. Positive Control: All (4/4) samples have to be positive
3. Samples: All (4/4) samples have to be negative

Subset 2:

1. NTC: All (2/2) NTCs have to be negative
2. Recovery Control: All (4/4) samples have to be positive

If the Recovery Control samples in Subset 2 show one or more negative results, the run is invalid. As a consequence, the entire run must be repeated.

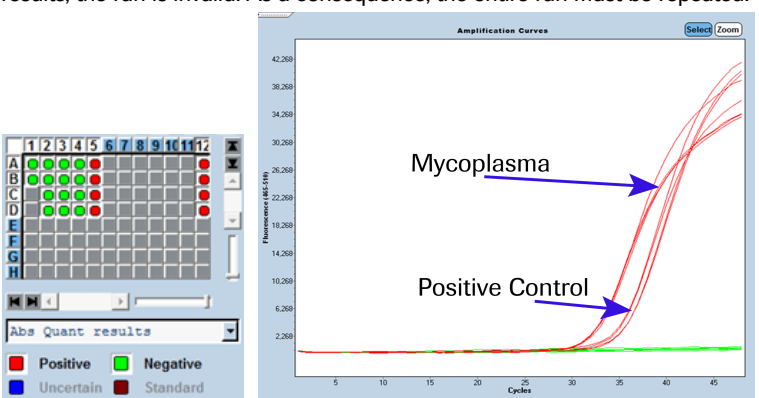


Fig. 4a: A typical analysis result (Mycoplasma PCR) (Subset 1): The plate view on the left shows whether a sample is negative (green) or positive (red). To the right, the amplification curves of samples and controls are shown.

NTC 1-2 (A1 + B1) = Negative Sample 3 (A4 - D4) = Negative
Sample 1 (A2 - D2) = Negative Sample 4 (A5 - D5) = 4/4 Positive
Sample 2 (A3 - D3) = Negative Positive Control (A12 - D12) = 4/4 Positive

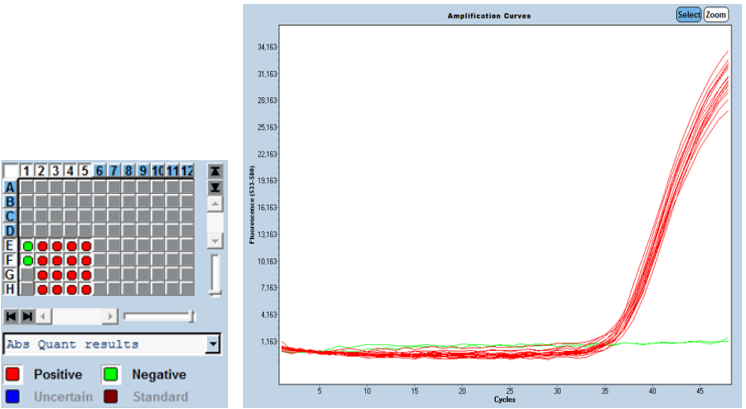


Fig. 4b: A typical analysis result (Recovery Control PCR) (Subset 2): The plate view on the left shows whether a recovery control sample is negative (green) or positive (red). To the right, the amplification curves of the recovery control samples are shown.

NTC 1-2 (E1 + F1) = Negative
Recovery Control Sample 1 (E2 - H2) = Positive
Recovery Control Sample 2 (E3 - H3) = Positive
Recovery Control Sample 3 (E4 - H4) = Positive
Recovery Control Sample 4 (E5 - H5) = Positive

In the above example, combining the results of Subset 1 and Subset 2 shows that Samples 1 to 3 are negative. This is because, the Mycoplasma PCRs shown in the plate schematic in Figure 4a are negative (green), and the respective Recovery Control PCRs are positive (red) in Figure 4b.

The above call is positive if the Mycoplasma PCRs shown in Figure 4a, and the respective Recovery Control PCRs shown in Figure 4b, have Cps of < 43.

3.2 PCR with the Applied Biosystems® 7500 Real-Time PCR System

The MycoTOOL Mycoplasma Real-Time PCR Kit has produced good results using an Applied Biosystems 7500 Real-Time PCR System for selected Mollicutes species. Instruments other than the LightCycler® Instrument may not make it possible to define a cut off Ct-value. Therefore the use of a real-time PCR instrument other than the LightCycler® 480 Instrument II will have to be evaluated by the user.

PCR Profile

Prepare Instrument according to the Operator's Manual.

Use the protocol as defined below:

Dye1/Quencher FAM/none
Dye2/Quencher VIC/none
Set up Sample Volume: 50 µl
Run Mode: Standard 7500

Program the protocol as indicated below and save it as a template file for reuse.

Stage	Step	Temp (°C)	Duration	Cycles
1	UNG Incubation	40	10:00 min	1
2	Initial Denaturation	95	10:00 min	1
3	Pre-Amplification	95 70 72	15 sec 15 sec 20 sec	2
4	Amplification	95 69	15 sec 35 sec	2
5	Amplification	95 68	15 sec 35 sec	2
6	Amplification	95 67	15 sec 35 sec	2
7	Amplification	95 66	15 sec 35 sec	2
8	Amplification	95 65	15 sec 35 sec	2
9	Amplification	95 64	15 sec 35 sec	2
10	Amplification	95 63	15 sec 35 sec	2
11	Amplification	95 62	15 sec 35 sec	2
12	Amplification	95 61	15 sec 35 sec	2
13	Amplification Data Collection	95 60	15 sec 35 sec	30

Data collection Stage 13, Step 2 (60.0 @ 0:35)

Make sure that the above protocol, including the touchdown steps is programmed. Runs performed with the instrument default protocol will produce invalid results.

Data Analysis

Perform data analysis according to the Applied Biosystems 7500 Real-Time PCR System Operator's Manual. Choose automated baseline and manual threshold. The threshold needs to be determined by the user. For the figures shown below, the threshold was set at 0.02.

⚠ The software of the Applied Biosystems 7500 Real-Time PCR System does not take into account the 18 touchdown cycles. This means that the Ct-value of 16 corresponds to a Cp-value (LightCycler® Instrument) of 34, as the 18 touchdown cycles are not added by the ABI Software during data analysis.

Result Interpretation

The real-time PCR amplification curve shows a positive result using specificity thresholds determined by the user. The same criteria as described in section 3.1 are used to designate a sample as negative or positive.

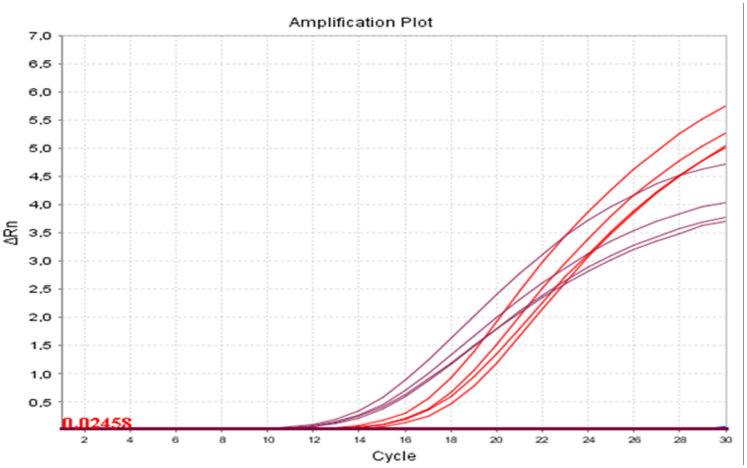


Fig. 5a: A typical analysis using the Applied Biosystems 7500 Real-Time PCR System in the fluorescence channel FAM. Red amplification curves correspond to the Positive Control. Purple amplification curves correspond to sample material positive for Mycoplasma. The Negative Control shows no amplification and is therefore negative.

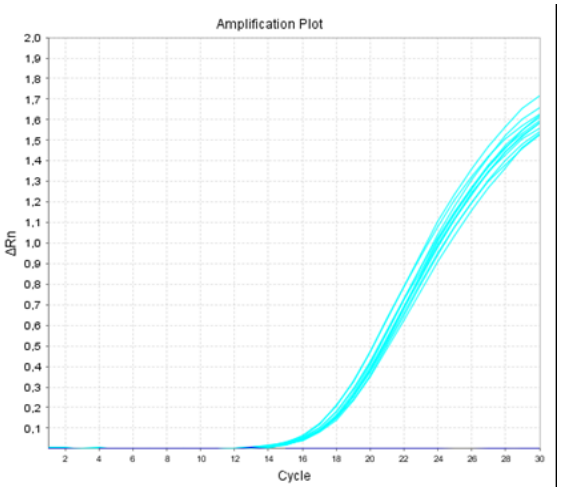


Fig. 5b: A typical analysis on an Applied Biosystems 7500 Real-Time PCR System in the fluorescence channel VIC. Turquoise amplification curves of the Recovery Control are positive. Negative control samples show no amplification, and are therefore negative.

4. Limitations

The kit was evaluated using the LightCycler® 480 Instrument II. Results obtained may also be valid for other real-time PCR instruments, but have to be verified empirically. In general, template concentration should not exceed 10 µg DNA/50 µl PCR.

Sensitivity of the MycoTOOL Mycoplasma Real-Time PCR Kit test using the MagNA Pure Instrument for purification is optimized for CHO cell cultures at cell densities up to 5×10^6 cells/ml. This kit is not recommended when working with higher cell densities.

Samples containing more than 5×10^6 CHO cells/ml should only be prepared using manual sample preparation. However, with such high cell densities, a partial PCR inhibition may be observed.

The MycoTOOL Mycoplasma Real-Time PCR Kit may also detect *Geobacillus sp*. contamination.

5. Troubleshooting

Guidelines for Working with Living Mycoplasma Strains

- Cultivation of Mycoplasma strains and determination of colony forming units (cfu) should meet the guidelines according to the European Pharmacopoeia, Chapter 2.6.7.
- For experiments in which Mycoplasma strains were used to infect cells, the ATCC materials recommended by the European Pharmacopoeia, Chapter 2.6.7., should be used.
- Local regulatory requirements for S2 laboratories should be adhered to.

6. Additional Information

6.1 Principle

Mycoplasma are severe contaminants in cell culture. Mycoplasma cell culture contamination occurs from individuals or contaminated cell culture medium ingredients. Mycoplasma induce cellular changes, including chromosome aberrations, changes in metabolism and cell growth. Severe Mycoplasma infections can destroy a cell line.

The kit uses specific PCR of highly conserved regions within Mycoplasma DNA. Highly specific primers and probes are included in the detection mix. Probes are labeled with a fluorescent dye detected by real-time PCR instruments. Primers are a mixture that principally allows the detection of more than 150 Mollicutes species, such as *A. laidlawii*, *M. fermentans*, *M. hyorhinis*, *M. orale*, *M. pneumoniae*, *M. synoviae*, *M. arginini*, *M. hominis*, *M. salivarium*, and *M. gallisepticum*.

The kit uses a ready-to-use hot start reaction mix for detecting DNA targets with hydrolysis probes. The chemically modified polymerase enzyme is inactive during initial PCR setup, thereby avoiding nonspecific elongation of primer template hybrids forming at lower temperatures. The polymerase is irreversibly activated by an initial activation step at higher temperature. To exclude false negative results, controls are included. Reagents are controlled by a positive control, and consists of a plasmid DNA (Positive Control). In addition, amplification of a second control plasmid (Recovery Control) added to the sample material, controls the efficiency of sample preparation, preventing false-negative results. The kit is designed to prevent PCR carryover contamination, using the provided Uracil-DNA Glycosylase (UNG). The incorporation of deoxyuridine tri-phosphate (dUTP) occurs during PCR, creating dUTP-containing amplicons. These can be digested by pretreatment of successive PCR mixtures with UNG. UNG removes uracil from DNA molecules by cleaving the N-glycosylic bond. Resulting abasic sites are hydrolyzed due to the high temperatures during the initial PCR denaturation step. Hydrolyzed DNA can no longer serve as a PCR template. UNG is inactivated during the initial denaturation step. Native DNA does not contain uracil, and is therefore not degraded by UNG-mediated denaturation.

The prevention of PCR carryover contamination is only possible using UNG, if the contaminating DNA contains uridine bases. Uridine containing DNA is produced by PCR if a nucleotide mix is applied that contains dUTP.

6.2 Quality Control

Each lot of this kit is function tested using the MagNA Pure 96 DNA and Viral NA Large Volume Kit with the MagNA Pure 96 and LightCycler® 480 Instrument II, using *A. laidlawii* at 3 and 10 cfu/ml in a 1 ml CHO cell culture with 5 × 10^6 cells/ml. In addition, the kit is tested for the absence of mycoplasmas.

6.3 Warranty

Roche Diagnostics warrants that these products will perform in accordance with manufacturer's specifications for the time period specified on the product packaging, when handled and stored in accordance with manufacturer's instruction stated herein. The sole and exclusive remedy for failure of products to meet specifications is refund of purchase price, repair or replacement, at manufacturer's sole option. ROCHE DIAGNOSTICS HEREBY DISCLAIMS ANY AND ALL OTHER WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR ANY INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

6.4 References

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7. Supplementary Information

7.1 Conventions

Text Conventions To make information consistent and easy to read, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ② *etc.*	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

7.2 Changes to Previous Version

- Updated regulatory disclaimer.
- New information added related to the REACH Annex XIV.
- Editorial changes.

7.3 Ordering Information

Product	Pack Size	Cat. No.
QC Sample Preparation Kit	1 Kit	08 146 829 001
LightCycler® 480 Instrument II	1 instrument 96-well version	05 015 278 001
LightCycler® 480 Multiwell Plate 96, white	5 × 10 plates with sealing foils	04 729 692 001
MagNA Pure 96 Instrument IVD	1 instrument, control unit and accessories	06 541 089 001
MagNA Pure 96 DNA and Viral NA Large Volume Kit	3 sets for 96 isolations each	06 374 891 001

7.4 Trademarks

MYCOTOOL, LIGHTCYCLER, and MAGNA PURE are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

7.5 Regulatory Disclaimer

For customers in the European Economic Area: Contains SVHC: octyl/nonylphenol ethoxylates. For use in analytical activities such as monitoring and quality control and under controlled conditions only– acc. to Art. 56 (3) and 3 no. 23 REACH Regulation.

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For more information about this product, as well as documentation such as Instructions for Use and Material Safety Data Sheets, please visit custombiotech.roche.com

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