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MycoTOOL Mycoplasma Real-Time PCR Kit

For the testing of the absence of Mycoplasma in CHO cells

Version 01

Content version: January 2012

REF 06 495 605 001

For the analysis of 10 samples by performing 160 PCR amplifications of 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 10 mammalian cell culture samples for the absence of Mycoplasma. The kit contains sufficient reagents to run 160 PCRs each with a 50 µl reaction volume.

Vial	Cap Color Code	Label	Content
1	blue	Recovery Control	1 vial, 200 µl (plasmid)
2	red	PCR Master, 2× conc.	5 vials, 1 ml each (2× concentrated master mix that contains all reagents – except primer and template – needed for running real-time DNA detection assays)
3	orange	UNG	1 vial, 180 µl (Enzyme that digests dUTP-containing DNA)
4	turquoise	PCR Enhancer	1 vial, 180 µl (PCR Additive)
5	green	Detection Mix, 25× conc.	1 vial, 220 µl (primers, FAM-labeled detection probe)
6	yellow	Detection Mix Recovery Control, 25× conc.	1 vial, 140 µl (primers, LightCycler® Yellow 555-labeled detection probe)
7	purple	Positive Control	1 vial, 800 µl (plasmid)
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 of the kit, store vial 5 and 6 protected from light.

Avoid repeated freezing and thawing of vial 2. After thawing, vial 2 may be stored for up to 4 weeks at +2 to +8°C

Assay Time

Hands on time PCR setup: approx. 1 hour.

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

Applications

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 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).

Assay Concept

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*, *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, 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 triphosphate (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.

Additional Equipment and Reagents Required

Additional equipment and reagents are required to perform nucleic acid isolation and amplification. To prepare the sample material for the analysis, you can choose an automated system using Roche's **MagNA Pure 96 Instrument** or a manual procedure using Roche's **MycoTOOL Mycoplasma Detection Prep Kit**.

For both types of sample preparation the following equipment is required:

- Pipettes
- Nuclease-free, DNA-free aerosol-resistant pipette tips
- Nuclease-free, DNA-free vials
- Alcohol wipes
- Biosafety cabinet class II

For automated sample preparation, the following additional equipment and reagents are required:

- MagNA Pure 96 Instrument (Cat. No. 05 195 322 001)
- MagNA Pure 96 DNA and Viral NA Large Volume Kit (Cat. No. 05 467 454 001)

For manual sample preparation, the following additional reagents are required:

- MycoTOOL Mycoplasma Detection Prep Kit (Cat. No. 05 184 592 001)

For the PCR workflow after sample preparation (reaction setup, amplification, analysis), the following equipment and reagents are required:

- 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 (Cat. No. 05 015 278 001, 96-well version)
- Multiwell Plates, (*e.g.,* LightCycler® 480 Multiwell Plate 96, white) for 50 µl PCRs
- Standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- Pipettes
- Nuclease-free, DNA-free aerosol-resistant pipette tips
- Nuclease-free, DNA-free vials for preparing PCR mixes
- Alcohol wipes
- Laminar flow hood

2. How to Use this Product

2.1 Special Handling

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

- Preparation and pipetting of all solutions with nuclease-free, DNA-free equipment and disposables
- UV-treatment of the laminar flow hood prior pipetting
- Use of sterile single-use gloves and wearing of freshly laundered laboratory coats
- Closing of vials immediately after pipetting
- Spatial segregation of the sequential workflow steps, which include sample preparation, master mix preparation, and final PCR setup and amplification run.

Perform sample preparation, master mix preparation and PCR run in separate locations.

Rooms	Workflow Step
Sample Preparation	Spike recovery control into sample. Set up and run automatic or manual sample preparation.
Master Mix Preparation	Pipette master mixes for sample analysis and controls.
PCR Setup and Amplification Run	Add samples and controls to PCR mix. Cover plate tightly with sealing foil. Centrifuge plate. Set up and start LightCycler® 480 Instrument II.

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.

2.2.1 Automated Sample Preparation

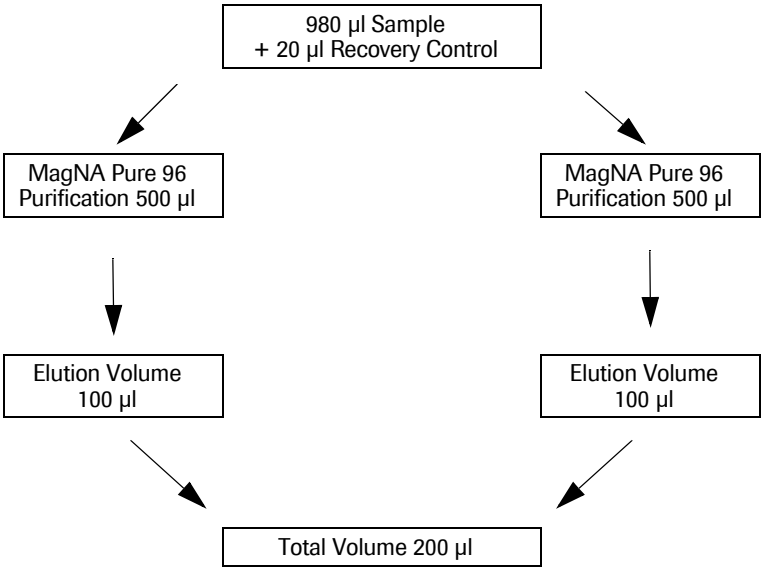
For automated sample preparation, use the MagNA Pure 96 Instrument (Cat. No. 05 195 322 001) with the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Cat. No. 05 467 454 001). Please refer to the corresponding manuals to make best use of this instrument and its reagents.

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 (Cat. No. 05 467 454 001) 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.

In addition to the guidelines provided in the respective MagNA Pure 96 Operator's Manual, do the following:

❶	Prepare 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 negative control and add 20 µl recovery control.
❻	Pipette split into 2 × 500 µl.
❼	Start the MagNA Pure 96 DNA purification run.
❽	DNA is eluted two times using 100 µl volume. Pool these two eluates.

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



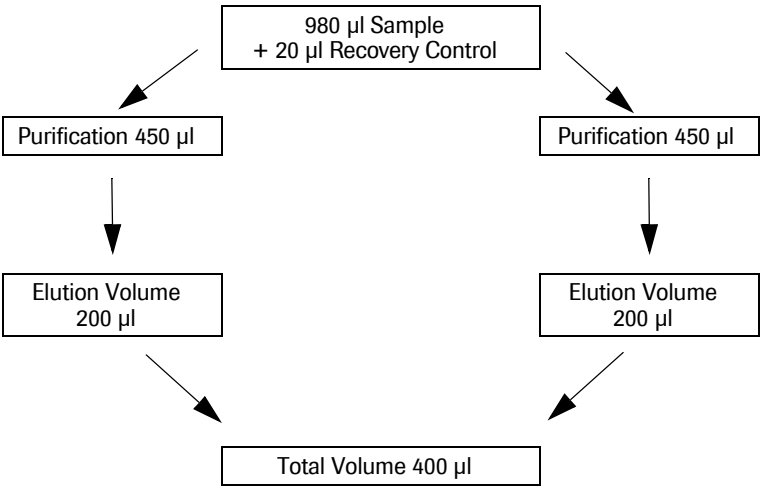
2.2.2 Manual Sample Preparation

For manual sample preparation, use the instructions of the MycoTOOL Mycoplasma Detection Prep Kit (Cat. No. 05 184 592 001). Adhere to the guidelines provided in this kit, by doing the following:

❶	Arrange reagents in the MycoTOOL Mycoplasma Detection Prep Kit.
❷	Equilibrate Thermomixer to +56°C.
❸	Take 980 µl sample or negative control (up to 5 × 10^6 cells/ml) and add 20 µl recovery control.
❹	Prepare two empty vials No. 6, (included in the MycoTOOL Mycoplasma Detection Prep Kit) with 30 µl Proteinase K (vial number 1) each. Label the vials accordingly.
❺	Add 450 µl sample to each vial.
❻	Add 450 µl Lysis Buffer (vial 2) to each vial.
❼	Close vial and vortex 3 × 5 s.
❽	Incubate for 15 min at +56°C/600 rpm in a pre-equilibrated thermomixer.
❾	Remove vials.
❿	Equilibrate thermomixer to +80°C.

⓫	Add 630 µl Precipitation Reagent (vial 3) to the vials.
⓬	Close vials, invert 20×, and vortex for 5 s.
⓭	Centrifuge for 3 min at 16,000 × *g*.
⓮	Decant supernatant without removing pellet.
⓯	Add 1 ml Washing Buffer (vial 4).
⓰	Close vials, invert 5×.
⓱	Centrifuge immediately for 3 min at 16,000 × *g*.
⓲	Remove supernatant quantitatively.
⓳	Centrifuge briefly for 3 s at 16,000 × *g*.
⓴	Take off again residual supernatant quantitatively.
㉑	Add 200 µl Dilution Reagent (vial 5).
㉒	Close vials and vortex briefly.
㉓	Dissolve pellet for 10 minutes at +80°C/900 rpm in a thermomixer.
㉔	Vortex until pellet is completely dissolved.
㉖	Pool the contents of the two DNA vials (2 × 200 µl). **
㉕	Transfer vials to the template preparation room.
㉗	Program instrument as described in Chapter 2.5. of this manual.

** DNA preparation is stable for 3 days at –15 to –25°C



2.3 Master Mix Preparation

Program your real-time PCR instrument as described in section 2.5.

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

Keep vial 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 down all other items with 70% ethanol before bringing into the hood.
❸	Place the reagents in a laminar flow hood, and let them thaw at room temperature (+15 to +25°C).
❹	Spin down briefly before opening.
❺	Change tip after each pipetting step.
❻	Prepare the two different Master Mixes, as described in section 2.3.2. Use nuclease-free, DNA-free vials.
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.

2.3.1 Number of PCR Reactions Required

For each sample to be tested, prepare the following number of PCRs. Run four replicates per PCR. Always run two negative controls (NTC) with the Mycoplasma PCR and with the Recovery Control PCR. To prepare a negative control (NTC), replace the template DNA with Water, PCR Grade (vial 8).

	Sample PCR rxn [1]	Positive Control rxn	NTC[2] Control rxn	Total rxn	Master Mix Preparation incl. 1 additional rxn	Master Mix [µl] 30 µl per rxn
Mycoplasma PCRs	4	4 [3]	2 [3]	10	11	330
Recovery Control PCRs	4	—	2 [3]	6	7	210

[1] rxn=reactions

[2] NTC= No template control (Water, PCR Grade, vial 8)

[3] Given is the number of reactions per plate

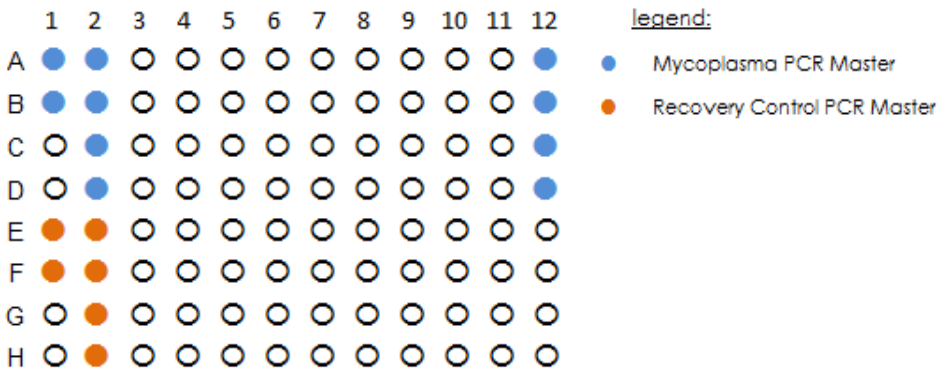


Fig. 1: Plate configuration proposal: One sample per plate. All wells colored in blue were selected in subset 1. All wells colored in orange were selected in subset 2 (see section 2.5.1.1).

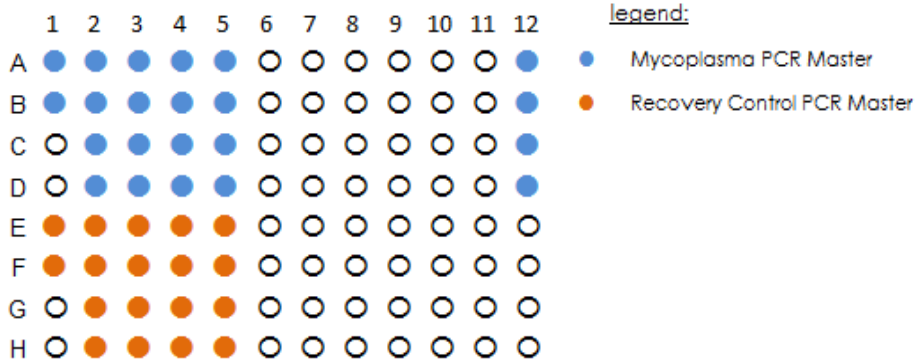


Fig. 2: Plate configuration proposal for more than one sample: *e.g.,* four samples per plate

2.3.2 Volume of Master Mix Required

The following volumes include one additional reaction for volume loss during pipetting.

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

* Calculate one additional reaction to compensate loss during pipetting (*e.g.,* single reaction = 30 µl, 10 reactions = 300 µl)

2.4 PCR Setup

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 water) to each well prefilled with master mix. Run replicates of each template as listed below.

	Sample	Positive Control	Negative Control	Number of PCR rxn
Mycoplasma PCRs	4 × 20 µl	4 × 20 µl	2 × 20 µl	10
Recovery Control PCRs	4 × 20 µl		2 × 20 µl	6

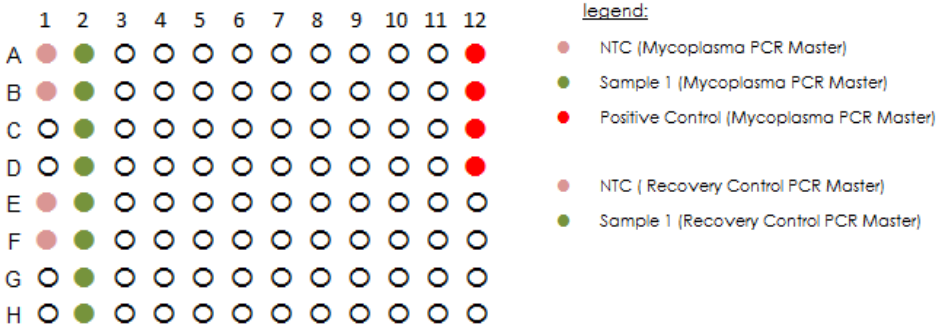


Fig. 3: Plate configuration proposal: One sample per plate

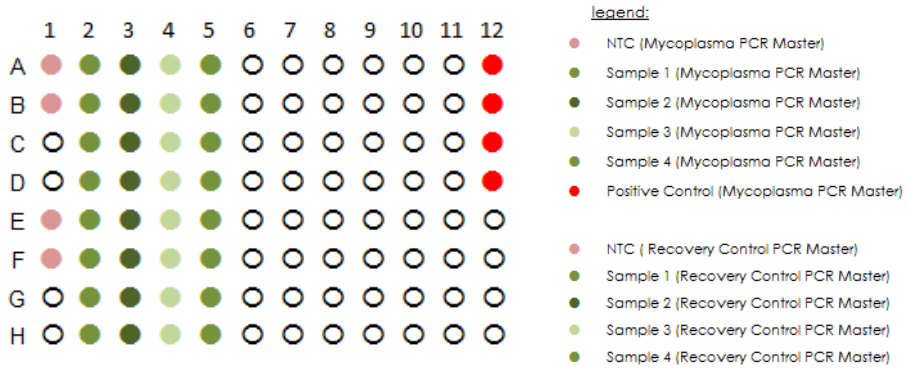


Fig. 4: Plate configuration proposal for more than one sample: *e.g.,* four samples per plate

❹	Seal the plate properly using a LightCycler® 480 Sealing Foil. Press it firmly to the plate surface using a scraper (*e.g.,* the Sealing Foil Applicator provided with the instrument). Remove side strips.
❺	Place the 96-well plate in a standard swing-bucket centrifuge, containing a rotor for 96-well plates with suitable adaptors. Balance it with a suitable counterweight (*e.g.,* another 96-well plate). Centrifuge the plate at 1,500 g for 2 minutes. Check the wells for bubbles, and repeat, if necessary.

2.5 Amplification by Real-Time PCR

Follow the general guidelines given in the manual of your real time PCR instrument. In addition, make sure that the settings are used, as indicated below. The temperature profile includes an initial incubation step at +40°C to allow UNG to digest dUTP containing DNA. The initial denaturation step will on one hand denature UNG and on the other hand activate the polymerase. Due to high genomic DNA background, a touchdown PCR protocol is required. To program a touchdown protocol on other than the LightCycler® 480 Instrument II, it may be necessary to modify interpretation of the data analysis accordingly (see chapter 2.5.2.2).

2.5.1 LightCycler® 480 Instrument II

2.5.1.1 Set up a PCR Profile

1. Start the LightCycler® 480 Instrument software.
2. Click on **Setup New Experiment**.
3. Use **Detection Format**: Dual Color Protocol Hydrolysis probe/ UPL probe.
4. Press “customize”, and check that FAM and VIC/ HEX/ Yellow555 are active.
5. Program the protocol as indicated below and save it as a template file for reuse.

Setup						
Detection Format	**Block Size**			**Reaction Volume**		
Dual Color Hydrolysis Probe/ UPL Probe	96			50 µl		
Programs						
Program Name	**Cycles**			**Analysis Mode**		
UNG	1			None		
Initial Denaturation	1			None		
Pre-Amplification	2			None		
Amplification	48			Quantification		
Cooling	1			None		
Temperatur Targets						
Target (°C)	**Aqusition 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	None	00:00:20	4.4	—	—	—
Cooling						
40	None	00:00:30	2.2	—	—	—

❻	Click **Subset Editor,** click on  button (new Subset 1), highlight the wells used for the Mycoplasma PCR (see figure above), and click the button **Apply**.
❼	Click **Subset Editor,** click on  button (new Subset 2), highlight the wells used for the Recovery Control PCR, and click the button **Apply**.
❽	Click **Sample Editor**. Choose new Subset 1 or new Subset 2, and provide the sample name(s). For replicate samples, click the **Replicate** button in the lower left corner.
❾	Load the LightCycler® 480 Instrument II with the prepared 96-well plate
❿	Start the PCR Program.

2.5.1.2 Data Analysis

❶	Click **Analysis**, "Create New Analysis" using Abs Quant/2nd Derivative Max for Subset 1 or Abs Quant/2nd Derivative Max for Subset 2, and select **Created Subset**.
❷	Turn on **Color Compensation** and select a **Color Compensation Object** from the database (Universal CC FAM(510)-VIC/ HEX /Yellow555(580) [465-510,533-580]).
❸	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.
❹	Click the **Safe** button in the Global action bar (on the right), to save the analysis results as part of the experiment.
❺	Click the **Calculate** button in the Global action bar (on the left), to save calculate the analysis results as part of the experiment.

2.5.1.3 Result Interpretation

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.

⚠ All samples with a Cp-value ≥ 43.0 are scored as negative. (Cut-Off Cp-value 43.0).

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.

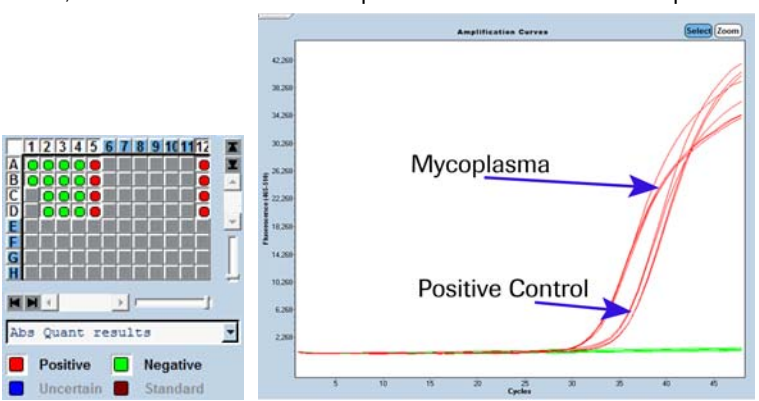


Fig. 5: A typical analysis result (Mycoplasma PCR) (Subset 1): The plate view on the left shows whether a sample is negative (green) or positive (red). To the right the amplification curves of samples and controls are shown.

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

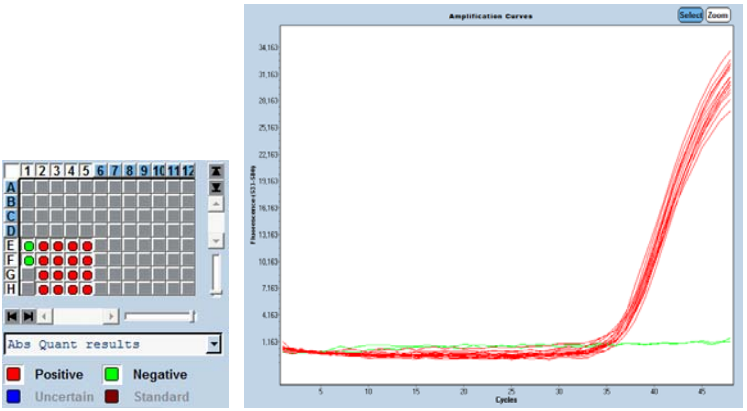


Fig. 6: A typical analysis result (Recovery Control PCR) (Subset 2): The plate view on the left shows whether a recovery control sample is negative (green) or positive (red). To the right the amplification curves of the recovery control samples are shown.

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 5 are negative (green), and the respective Recovery Control PCRs are positive (red) in Figure 6.

To make the above call valid, it is essential to verify that the Mycoplasma PCRs shown in Figure 5, and the respective Recovery Control PCRs shown in Figure 6, have Cps of less than 43, to be called positive.

2.5.2 Applied Biosystems® 7500 Real-Time PCR System

The 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 validated by the user.

2.5.2.1 Setup and PCR Profile using an Applied Biosystems 7500 Real-Time PCR System

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.

2.5.2.2 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.

2.5.2.3 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 2.5.1.3 are used to designate a sample as negative or positive.

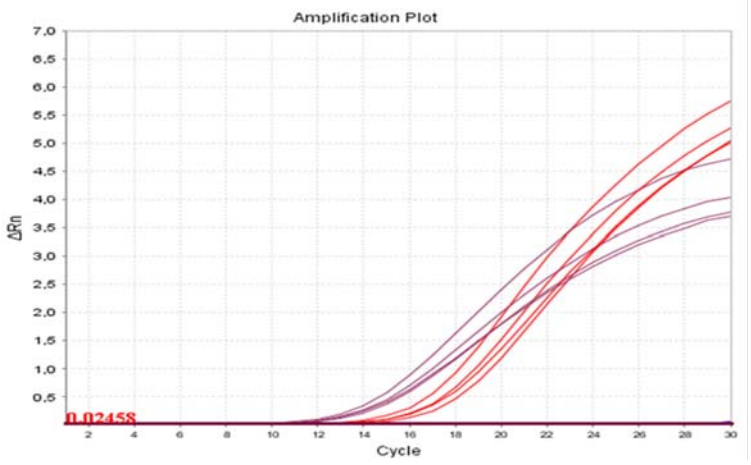


Fig. 7: A typical analysis using the Applied Biosystems 7500 Real-Time PCR System in the fluorescence channel FAM. Red amplification curves correspond to the Positive Control. Purple amplification curves correspond to sample material positive for Mycoplasma. The Negative Control shows no amplification, and is therefore negative.

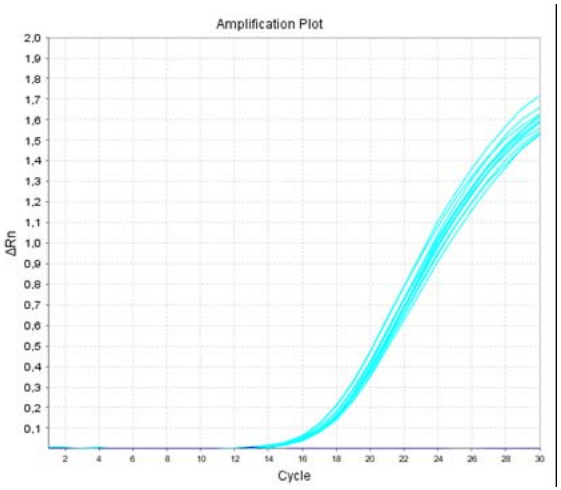


Fig. 8: A typical analysis on an Applied Biosystems 7500 Real-Time PCR System in the fluorescence channel VIC. Turquoise amplification curves of the Recovery Control are positive. Negative control samples show no amplification, and are therefore negative.

3. 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.

Cell-free samples cannot be tested due to limitations in sample preparation.

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

4. Troubleshooting

4.1 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.

5. Additional Information

5.1 Specificity

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.

5.2 Sensitivity

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

5.3 Quality Control

Each lot of this kit is function tested using the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Cat. No. 05 467 454 001) 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.

5.4 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 sold 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.

5.5 References

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6. Supplementary Information

6.1 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 Applied Science.

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.

6.2 Changes to Previous Version

This is the first version.

6.3 Supplementary Information

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research.

For a complete overview of related products and manuals, please visit and bookmark our Homepage, http:\\www.roche-applied-science.com, and our Special Interest Sites including:

- Amplification - Innovative Tools for PCR:
  http://www.roche-applied-science.com/pcr
- DNA & RNA preparation – Versatile Tools for Nucleic Acid Purification:
  http://www.roche-applied-science.com/napure
- The MagNA Pure System family for automated nucleic acid isolation:
  http://www.magnapure.com.

Product	Pack Size	Cat. No.
MycoTOOL Mycoplasma Real-Time PCR Kit	10 samples, 160 PCR	06 495 605 001
MycoTOOL Mycoplasma Detection Prep Kit	20 preparations	05 184 592 001
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	1 instrument, control unit and accessories	05 195 322 001
MagNA Pure 96 DNA and Viral NA Large Volume Kit	3 sets for 96 isolations each	05 467 454 001
MVM PCR Detection Kit	Kit for 10 samples, 30 PCR	06 479 359 001

6.4 Trademarks

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

6.5 Regulatory Disclaimer

For use in quality control/manufacturing process only.

6.6 License Disclaimer

NOTICE: This product is subject to certain use restrictions. Before using this product please refer to Patent and License Disclaimer number 49 (https://www.roche-applied-science.com/new/legal/index.jsp?id=legal_000000) for current information concerning FastStart Taq DNA Polymerase. Further restrictions may apply. License Disclaimer information is subject to change or amendment.

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