

# Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM

## Handbook

Real Time PCR kit for qualitative detection of  
*Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*

**REF** B42-4-50FRT

 **50**

## NAME

### **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM**

## INTRODUCTION

***Mycoplasma pneumoniae*** is spread through respiratory droplet transmission. Once attached to the mucosa of a host organism, *M. pneumoniae* extracts nutrients, grows and reproduces. Attachment sites include the upper and lower respiratory tract, causing pharyngitis, bronchitis, and pneumonia. The infection caused by this bacterium is called atypical pneumonia because of its protracted course and lack of sputum production and wealth of extra-pulmonary symptoms.

**Chlamydophila** (formerly Chlamydia) **pneumoniae** causes mild pneumonia or bronchitis in adolescents and young adults. Older adults may experience more severe disease and repeated infections. Approximately 50% of young adults and 75% of elderly persons have serological evidence of previous infection. The pathogen is estimated to cause 10-20% of community-acquired pneumonia cases among adults. The estimated number of cases of *C. pneumoniae* pneumonia is 300,000 cases per year.

## INTENDED USE

Kit **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM** is a “Real-Time Amplification” test for the qualitative detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in the biological materials (whole blood, tissue, swabs, etc).

## PRINCIPLE OF ASSAY

Kit **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM** is based on two major processes: DNA is extracted from samples and amplified using real time amplification with fluorescent reporter dye probes specific for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and Internal Control IC. Test detects an endogenous IC of a human genome DNA fragment which is extracted from the sample and serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. IC is detected in a channel other than the *M.pneumoniae* or *C. pneumoniae*.

## MATERIALS PROVIDED

### “*Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae* Real-TM”:

- **PCR-mix-1**, 55 ready-to-use single-dose test tubes;
- **PCR-mix-2-Flu**, 0,77 ml;
- **Positive Control *Chl. Pneumoniae*/*Myc pneumoniae*/Prothrombin C+**, 0,1 ml;
- **Negative Control C-\***, 1,2 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 55 tests.

\* must be used in the extraction procedure as Negative Control of Extraction

## MATERIALS REQUIRED BUT NOT PROVIDED

- Real Time Thermal cycler
- Reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Vortex mixer
- Freezer, refrigerator

## STORAGE INSTRUCTIONS

***Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae* Real-TM** must be stored at 2-8°C.

The kits can be shipped at 2-8°C but should be stored at 2-8°C immediately on receipt.

## STABILITY

***Mycoplasma pneumoniae* / *Chlamydomphila pneumoniae* Real-TM** is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided.

## QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

## WARNINGS AND PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

## PRODUCT USE LIMITATIONS

Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

## SAMPLE COLLECTION, STORAGE AND TRANSPORT

**Mycoplasma pneumoniae / Chlamydomphila pneumoniae Real-TM** can analyze DNA extracted from:

- *Whole blood* collected blood in ACD or EDTA tubes;
- *tissue* (≈1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile. Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- *bronchial lavage*: centrifuge 10 mL at 3000 g/min for 10-15 min. Remove and discard the supernatant. If the pellet isn't visible add 10 ml of liquid and repeat centrifugation remove and discard the supernatant. Resuspend the pellet in 100 µl of saline water.
- *swabs*: insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Specimens can be stored at +2-8°C for no longer than 12 hours, or freeze at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

## DNA ISOLATION

The following kit is recommended:

- ⇒ **DNA-Sorb-B** (Sacace, REF K-1-1/B);
- ⇒ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006);

Please carry out DNA extraction according to the manufacturer's instruction.

## PROTOCOL (Reaction volume 25 µl):

1. Prepare required quantity of **PCR-mix-1** tubes for samples and controls.
2. Add **7 µl** of **PCR-mix-2 Flu** into each tube.
3. Add **10 µl** of **extracted DNA** sample to appropriate tube.
4. Prepare for each panel 4 controls:
  - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
  - add **10 µl** of **Positive Control** *Chl. Pneumoniae/ Myc pneumoniae/Prothrombin* to the tube labeled C+;

## Amplification

1. Create a temperature profile on your instrument as follows:

Step	Rotor-type Instruments <sup>1</sup>			Plate- or modular type Instruments <sup>2</sup>		
	Temperature, °C	Time	Repeats	Temperature, °C	Time	Repeats
1	95	5 min	1	95	5 min	1
2	95	10 s	10	95	10 s	10
	60	20 s		60	25 s	
	72	10 s		72	25 s	
3	95	10 s	35	95	10 s	35
	60	20 s <i>fluorescent signal detection</i>		60	25 s <i>fluorescent signal detection</i>	
	72	10 s		72	25 s	

<sup>1</sup> For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)

<sup>2</sup> For example, SaCycler-96™ (Sacace), CFX96/iQ5™ (Biorad), Mx3000P/3005P™ (Agilent)

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in Fam (Green), Rox (Orange) and Joe (Yellow), fluorescence channels.

*Mycoplasma pneumoniae* is detected on the FAM (Green) channel, *Chlamydomphila pneumoniae* on ROX (Orange) and human DNA on the JOE(Yellow)/HEX/Cy3 channel.

## INSTRUMENT SETTINGS

### Rotor-type instruments

Channel	Calibrate/Gain Optimisation...	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	<i>from 5 FI to 10 FI</i>	0.05	5 %	Off
ROX/Orange	<i>from 5 FI to 10 FI</i>	0.1	5%	Off
JOE/Yellow	<i>from 5 FI to 10 FI</i>	0.1	0 %	Off

### Plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

### Results for controls

Control	Stage for control	Ct channel Fam/Green	Ct channel Joe/Yellow	Ct channel Rox/Orange	Interpretation
NCE	DNA isolation	Neg	Neg	Neg	Valid result
NCA	Amplification	Neg	Neg	Neg	Valid result
Pos C+	Amplification	Pos (< 33)	Pos (< 31)	Pos (< 33)	Valid result

## PERFORMANCE CHARACTERISTICS

### Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* primers and probes. The specificity of the kit **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM** was 100%. The potential cross-reactivity of the kit **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

### Analytical sensitivity

The kit **Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM** allows to detect *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

**Target region:** *Mycoplasma*: putative lipoprotein; *Chlamydophila*- ompA

## TROUBLESHOOTING

### 1. Weak or no signal of the IC.

- The PCR was inhibited.
  - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
  - ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
- The reagents storage conditions didn't comply with the instructions.
  - ⇒ Check the storage conditions
- The PCR conditions didn't comply with the instructions.
  - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.

### 2. Weak or no signal of the Positive Control.

- The PCR conditions didn't comply with the instructions.
  - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.

### 3. FAM (Green) or Rox (Orange) signal with Negative Control of extraction.

- Contamination during DNA extraction procedure. All samples results are invalid.
  - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
  - ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
  - ⇒ Repeat the DNA extraction with the new set of reagents.

### 4. Any signal with Negative Control of PCR (DNA-buffer).

- Contamination during PCR preparation procedure. All samples results are invalid.
  - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
  - ⇒ Pipette the Positive control at last.
  - ⇒ Repeat the PCR preparation with the new set of reagents.

## KEY TO SYMBOLS USED



List Number



Caution!



Lot Number



Contains sufficient  
for <n> tests



Store at



Version



Manufacturer

**NCA**

Negative Control of  
Amplification



Consult instructions for  
use

**NCE**

Negative control of  
Extraction



Expiration Date

**C+**

Positive Control of  
Amplification

**RUO**

For Research Use Only

**IC**

Internal Control

\* SaCycler™ is a registered trademark of Sacace Biotechnologies

\* Rotor-Gene™ is a registered trademark of Qiagen

\* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories

\* MX 3000P/3005P® is a registered trademark of Agilent Technologies



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