FOR *IN VITRO* USE ONLY

microproof® *Streptococcus pneumoniae* Detection Kit
– Hybridization Probes (LC 2.0) –

Version 4, September 2017

PCR kit for the qualitative detection of *Streptococcus pneumoniae* DNA using the LightCycler® 2.0 Carousel-Based System.

Order No. R 300 32

PCR Kit for 96 reactions

Store at -15 to -25 °C

Table of Contents

1 Kit Components

Number of Tests

The **micro**proof® *Streptococcus pneumoniae* Detection Kit is designed for:

- 96 reactions
- a reaction volume of 20 µl
- up to 30 samples (single sample preparation) plus positive and negative control reactions can be analyzed per LightCycler® 2.0 Carousel-Based System run (i.e., the complete system allows analysis of a maximum of 90 samples).

Storage and Stability

- Store at –15 °C to –25 °C through the expiration date printed on the label.
- Once opened, store the components as described in the following Contents table:

Kit Contents

Vial/Cap Color	Label	Contents / Function / Storage
1 yellow cap	*Streptococcus pneumoniae* Detection Kit - Master Mix -	• 3 x 420 µl • Ready-to-use primer and Hybridization Probe mix for the specific amplification and detection of DNA of *Streptococcus pneumoniae* and the Internal Control (IC). • Store at -15 to - 25°C. • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 white cap	*Streptococcus pneumoniae* Detection Kit - Internal Control -	• 3 x 32 µl • Contains a stabilized solution of plasmid DNA. • For use as an internal amplification control. • Store at -15 to -25°C. • After first thawing store at +2 °C to +8 °C for up to one month.
3 red cap	*Streptococcus pneumoniae* Detection Kit - Enzyme Solution -	• 3 x 32 µl • Contains Taq DNA Polymerase and Uracil-DNA Glycosylase (heat labile) for prevention of carry-over contamination. • Store at -15 to -25°C.
4 purple cap	*Streptococcus pneumoniae* Detection Kit - Control Template -	• 2 x 60 µl • Contains a stabilized solution of plasmid DNA. • For use as a PCR run positive control. • Store at -15 to -25°C. • After first thawing store at +2 °C to +8 °C for up to one month.
5 colorless cap	*Streptococcus pneumoniae* Detection Kit - H_2O, PCR-grade -	• 1 x 1 ml • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control. • Store at -15 to -25°C.

Additional Equipment and Reagents Required

- LightCycler® Carousel-Based System (LightCycler® 2.0 Instrument, Roche Applied Science)[1]
- LightCycler® 20 µl – Capillaries[1]
- Color Compensation Set 4 (Order No. A 500 11)[2]
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.
- The LightCycler® 2.0 Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.
- or
- LC Carousel Centrifuge 2.0[1] for use with the LightCycler® 2.0 Sample Carousel (optional).
- **micro**proof® Suspension Buffer (Order No. S 400 10)[2]

or

- **food**proof® ShortPrep II Kit (Order No. S 400 02)[2]
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions

[1] Available from Roche Diagnostics
[2] Available from BIOTECON Diagnostics; see Ordering Information for details

Applicability Statement

The **micro**proof® *Streptococcus pneumoniae* Detection Kit is designed for the rapid detection of microorganisms of the species *Streptococcus pneumoniae*.
The **micro**proof® *Streptococcus pneumoniae* Detection Kit must not be used in diagnostic procedures.
The detection kit described in this Instruction Manual has been developed for the LightCycler® 2.0 Carousel-Based System.

Assay Time

Procedure	Time
PCR-setup	15 min
LightCycler® carousel-based system PCR run	60 min
Total assay time	75 min

2 How to Use this Product

2.1 Before You Begin

Precautions

Detection and identification of target DNA using the **micro**proof® *Streptococcus pneumoniae* Detection Kit requires DNA amplification by PCR. The detection kit provides all the reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease, carry-over, or cross-contamination:

- Prepare appropriate aliquots of the solutions and keep them separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.
- Physically separate the workplaces for DNA preparation, PCR-setup, and PCR to minimize the risk of carry-over contamination. Use a PCR-hood for all pipetting steps.
- In order to avoid cross-contamination, close all capillaries that contain sample DNA and negative controls before pipetting positive controls.

Waste Disposal

Place any waste and biohazard material potentially contaminated with pathogenic bacteria in an appropriate plastic Contaminated Waste bag and label as follows: CONTAMINATED Waste, Room number, date and initials. The bag should be autoclaved and then disposed of according to local regulations.

Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For rapid testing of colonies from agar plates or from enrichment cultures the use of the Suspension Buffer or the **food**proof® ShortPrep II Kit is recommended (see Additional Reagents Required).

Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [*Streptococcus pneumoniae* Detection Kit Positive Control (vial 4, purple cap)]. Always close capillaries with template DNA and negative controls before adding positive control DNA.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O, PCR-grade (vial 5, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.2 Procedure

The described procedure is optimized for use with the LightCycler® 2.0 Carousel-Based System. Program the LightCycler® Carousel-Based System with the following time-temperature protocol before preparing the working solutions (for details on how to program the experimental protocol and how to generate an Experiment Kit Macro, refer to the LightCycler® 2.0 Instrument Operator's Manual):

Program Name	Cycles	Analysis Mode
Preincubation	1	None
Amplification	45	Quantification
Melting Curve Analysis	1	Melting Curves
Cooling	1	None

Program	Target [°C]	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Sec Target [°C]	Step Size [°C]	Step Delay [cycles]	Acquisition Mode
Preincubation							
Segment 1	37	00:04:00	20	0	0	0	None
Segment 2	95	00:05:00	20	0	0	0	None
Amplification							
Segment 1	95	00:00:05	20	0	0	0	None
Segment 2	60	00:00:20	20	0	0	0	Single
Segment 3	72	00:00:10	20	0	0	0	None
Melting Curve Analysis							
Segment 1	95	00:00:05	20	0	0	0	None
Segment 2	40	00:01:00	20	0	0	0	None
Segment 3	75	00:00:00	0,1	0	0	0	Continuous
Cooling							
Segment 1	40	00:00:30	20	0	0	0	None

Note: The kit can also be used with the protocol of the **micro**proof® *Streptococcus pneumoniae* Identification Kit.

Fluorescence and Run Setup Parameters

Parameter	Setting
Default Channel • during run: • for analysis:	 • 670 or 705 • Refer to 2.3.Analysis
Seek Temperature	30°C
Max. Seek Pos	Enter the number of samples including controls
Instrument Type	6 Ch.
Capillary Size	20µl

Preparation of the PCR Mix

Note:	**Wear gloves when handling the capillaries – do not touch the surface**

Step 1: Depending on the total number of reactions, place the required number of LightCycler® Capillaries in the centrifuge adapters or in a LightCycler® Sample Carousel in a LightCycler® Carousel Centrifuge Bucket

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Step 2: Thaw the solutions and briefly spin vials in a microcentrifuge before opening.
Mix carefully but thoroughly by pipetting up and down.

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Step 3: In a 1.5 ml reaction tube prepare the PCR mix by adding the following components in the order mentioned below, and then mix gently by pipetting up and down.
Prepare the PCR Mix by multiplying the amount in the "Volume" column by the number of reactions to be cycled plus one or two additional reactions to cover pipetting losses

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Component			**Volume**
Vial 1	yellow cap	- Master Mix -	13µl
Vial 2	white cap	- Internal Control -	1µl
Vial 3	red cap	- Enzyme Solution -	1µl
		Sample	5µl
		Total volume	20µl

Note:	**Mix carefully but thoroughly by pipetting up and down. Do not vortex.**

Step 4: Pipet 15 µl PCR mix into each LightCycler® capillary

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Step 5: Sample DNA: Add 5 µl to a capillary, seal with a stopper

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Step 6: Negative Control: Add 5 µl H_2O, PCR-grade (vial 5, colorless cap) to a capillary, seal with a stopper

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Step 7: Positive Control: Add 5 µl H_2O, PCR-grade (vial 4, purple cap) to a capillary, seal with a stopper

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Step 8: Place the adapters (containing the capillaries) in a standard benchtop microcentrifuge. (Place the centrifuge adapters in a balanced arrangement within the centrifuge.) Centrifuge at 700 x *g* for 5 s (3,000 rpm in a standard benchtop microcentrifuge). Alternatively, use the LightCycler® Carousel Centrifuge for spinning the capillaries.

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Step 9: Transfer the capillaries to the LightCycler®

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Step 10: Cycle the samples as described above

2.3 Analysis

Different fluorescence channels are used to monitor the amplification of **DNA of *Streptococcus pneumoniae* (channel 670)** and the specific amplification of the **Internal Control (channel 705)**.

Color Compensation

The use of a previously generated system-specific color compensation object is a prerequisite for the analysis to compensate for the crosstalk between the detection channels 530, 670, and 705. For additional information on the generation and use of a color compensation object, refer to the LightCycler® Instrument Operator's Manual.

1. Add the **analysis module**, click **Color Compensation** in the analysis window, than select **Select Color Compensation**.
2. Select the color compensation object you want to apply, and then click **OK**.
3. A small dialog box opens so you can **select the channels to compensate**. The number of channels displayed depends on the number of channels used in the color compensation experiment. By default all channels are selected.
4. **Deselect any channels you do not want to compensate** (i.e., for this system select channels 530, 670 and 705) then click **OK**.
5. The analysis charts are redrawn using the compensated data. Note that the Color Compensation menu label now says "(On)".

Note: Analysis templates including Color Compensation objects can be created from analysis modules to reduce time and effort for analysis (refer to the LightCycler® 2.0 Instrument Operator's Manual).

Interpretation of Amplification Curves

Compare the results from channel 670, and channel 705 (Internal Control) for each sample, and interpret as described in the following table.

670	705	Result Interpretation
+	+ or -	positive for *Streptococcus pneumoniae*
-	+	negative for *Streptococcus pneumoniae*
-	-	invalid

+ / - : Positive or negative for amplification. Check the results of the software visually for plausibility.

Specificity

The *Streptococcus pneumoniae* Detection Kit Master Mix is sequence-specific for *Streptococcus pneumoniae*. Inclusivity has been tested with more than 90 strains of *Streptococcus pneumoniae* including all known serotypes. Exclusivity was determined using more than 60 species of closely related organisms (e.g. *Streptococcus pseudopneumoniae*) or organisms occurring in the same habitat.

3 Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set Channel settings to 670 or 705. Fluorescence data is acquired for all channels during the run, regardless of the channel settings. If the incorrect channel is selected, there is NO need to abort and redo the run.
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs. • Select acquisition mode “single” at the end of each annealing segment of the PCR program.
No signal increase in channel 705 is observed.	Inhibitory effects of the sample material (*e.g.* caused by too much cell material or DNA).	• Use the recommended sample preparation. • Dilute sample extracts (*e.g.* in H_2O, PCR-Grade, or Suspension Buffer).
Fluorescence intensity is too low.	Inappropriate storage of components.	• Store the *Streptococcus pneumoniae* Detection Kit Master Mix (vial 1, yellow cap) at -15 °C to –25 °C, protected from light. • Avoid repeated freezing and thawing.
	Streptococcus pneumoniae Detection Kit Master Mix (vial 1, yellow cap) is not homogeneously mixed.	• Mix the *Streptococcus pneumoniae* Detection Kit Master Mix (vial 1, yellow cap) thoroughly before pipetting.
	Too high initial amount of target DNA.	• Dilute sample extracts (*e.g.* in H_2O, PCR-Grade, or Suspension Buffer).
Negative control samples are positive.	Carry-over contamination.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, reagents and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive controls after sample capillaries and negative control capillaries have been sealed with stoppers.
Fluorescence intensity varies.	Insufficient centrifugation of the capillaries. Prepared PCR mix is still in the upper vessel of the capillary. Air bubble is trapped in the capillary tip.	Always centrifuge capillaries (loaded with the reaction mix) as described.
	Outer surface of the capillary tip is dirty (*e.g.,* by direct skin contact).	Always wear gloves when handling the capillaries.

4 Additional Information on this Product

How this Product Works

The **micro**proof® *Streptococcus pneumoniae* Detection Kit provides primers and Hybridization Probes (for sequence-specific detection), convenient premixed reagents, and a Positive Control for reliable interpretations of results. To ensure maximum reliability of the reagents and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is supplied with the detection system (vial 2, white cap). The IC has to be added to each reaction. Hybridization Probes were designed to bind specifically the IC, allowing detection in channel 705, whereas the target DNA is detected in channel 670. In case of a negative result due to inhibition of amplification by the sample extract of interest, the amplification of the IC is suppressed as well. Whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of target DNA in the sample. The **micro**proof® *Streptococcus pneumoniae* Detection Kit minimizes contamination risk and contains all reagents needed. The detection system is specifically adapted for PCR in glass capillaries using the LightCycler® 2.0 Carousel-Based System. The detection system described in this Instruction Manual has been developed for the LightCycler® 2.0 Carousel-Based System.

Test Principle

1. Using the supplied sequence-specific primers in a polymerase chain reaction (PCR), the LightCycler® 2.0 Carousel- Based System and its associated reagents amplify and simultaneously detect fragments of genomic DNA of the target organisms.
2. The LightCycler® 2.0 Carousel-Based System detects these amplified fragments in real time through fluorescence generated by their corresponding pair of sequence-specific Hybridization Probes. For each amplicon, one probe is labeled at the 5´-end with an acceptor fluorophore and, to avoid extension, is modified at the 3´-end by phosphorylation. The other oligonucleotide probe is labeled at the 3´-end with a donor fluorophore.

3. During the annealing phase of each PCR cycle, these probes hybridize to an internal sequence of the amplicon. Only while hybridized in close proximity to each other do these probes result in fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, the light source of the LightCycler® Carousel-Based System excites the donor fluorophore and part of the excitation energy is transferred to the acceptor fluorophore.
4. The LightCycler® Instrument measures the emitted fluorescence of the acceptor fluorophore.

Quality Control

The **micro**proof® *Streptococcus pneumoniae* Detection Kit is function tested using the LightCycler® 2.0 Carousel-Based System.

5 Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under U.S. Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008. Email: outlicensing@lifetech.com.

5.3 Trademarks

LIGHTCYCLER and HYBPROBE are trademarks of Roche. Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions or experience problems with this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

6 Change Index

Version 1,
First version of the package insert.

Version 2, February 2013
A second control template (tube 4, purple cap) has been added to the kit, and the volume of each control template tube has been increased from 50 µl to 60 µl.

Version 3, March 2017
License Notice changed.

Version 4, September 2017
License Notice changed.

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