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

Order No. R 300 32

Quick Reference Procedure

Version 1, March 2012

A. LightCycler® Carousel-Based System Protocol

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	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

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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

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

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.

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

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

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

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

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.

Step 9: Transfer the capillaries to the LightCycler®

Step 10: Cycle the samples as described above

***Note:** For further information please refer to: http://www.bc-diagnostics.com/?cid=1327572117&lang=1

BIOTECON Diagnostics GmbH
Hermannswerder 17,
14473 Potsdam – Germany
Phone +49 (0) 331 2300-200, Fax +49 (0) 331 2300-299
bcd@bc-diagnostics.com, www.bc-diagnostics.com

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