FOR *IN VITRO* USE ONLY.

microproof® Hygiene Screening System

(*Staphylococcus, Micrococcus, Corynebacterium*)

- Hybridization Probes (LC 2.0) -

Version 4, March 2017

PCR system for the qualitative detection of *Staphylococcus, Micrococcus,* and *Corynebacterium* DNA, including the simultaneous identification of *Staphylococcus aureus* and *Micrococcus luteus*, using the LightCycler® 2.0 Carousel-Based System

Order No. R 300 31

PCR system for 96 reactions

Store at -15 to -25 °C

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1. What this Product Does

Number of Tests

The **micro**proof Hygiene Screening System is designed for 96 reactions with a final reaction volume of 20 µl each. 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 the kit at –15 °C to –25 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Contents table:

Kit Contents

Vial	Label	Contents / Function / Storage
1 yellow cap	**micro**proof Hygiene Screening System Master Mix	• 3 x 520 µl • Ready-to-use primer and Hybridization Probe mix for the specific amplification and detection of DNA of *Staphylococcus, Micrococcus, Corynebacterium*, and the Internal Control (IC). • Store at -15 to – 25 °C. • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 white cap	**micro**proof Hygiene Screening System 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 purple cap	**micro**proof Hygiene Screening System Control Template	• 1 x 50 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.
4 colorless cap	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)[2]
- LightCycler® 20 µl - capillaries[2]
- **micro**proof Hygiene Screening System Color Compensation Set 4[1]
- 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[2] for use with the LightCycler® 2.0 Sample Carousel (optional).
- Suspension Buffer[1]
- HotStart Taq polymerase, *e.g.* **micro**proof Enzyme Mix (Taq Polymerase/UNG)[1]
  (the **micro**proof Hygiene Screening System is validated with this Enzyme Mix only)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions

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

Applicability Statement

The **micro**proof Hygiene Screening System is designed for the rapid identification of microorganisms of the genera *Corynebacterium, Staphylococcus, Macrococcus, Micrococcus, Kocuria*, and *Kytococcus*. Furthermore, some coryneform bacteria and *Nesterenkonia halobia* are detected. A melting curve analysis allows further differentiation and identification of some species and groups of species.

The **micro**proof Hygiene Screening System must not be used in diagnostic procedures.
It does not contain Taq Polymerase (see Additional Equipment and Reagents required).

The detection system 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	50 min
Total assay time	65 min

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection and identification of target DNA using the **micro**proof Hygiene Screening System requires DNA amplification by PCR. The detection system provides all the reagents required for the PCR except for Taq polymerase (see Additional Equipment and Reagents required). 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 the use of Suspension Buffer 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 [Hygiene Screening System Positive Control (vial 3, 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 4, 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 following procedure is based on the use of **micro**proof Enzyme Mix (Taq Polymerase / UNG) from BIOTECON Diagnostics. Use of a different Taq polymerase and / or Uracil-DNA-Glycosylase brand might result in changes of the pre-incubation steps of the PCR program and of the pipetting scheme.

LightCycler® 2.0 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):

Pre-incubation (prevention of carry-over contamination, activation of Taq DNA polymerase, denaturation of template DNA)		
Programs/Cycle Program Data	***Value***	
Cycles	1	
Analysis Mode	None	
Temperature Targets	***Segment 1***	***Segment 2***
Target/Target Temperature [°C]	40	95
Hold/Incubation Time [h:min:s]	00:02:00	00:02:00
Ramp Rate/Temperature Transition Rate [°C/s]	20	20
Sec Target/Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [cycles]	0	0
Acquisition Mode	None	None

Amplification (of the target DNA)			
Programs/Cycle Program Data	***Value***		
Cycles	35		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	62	72
Hold/Incubation Time [h:min:s]	00:00:02	00:00:20	00:00:10
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	20
Sec Target/Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [cycles]	0	0	0
Acquisition Mode	None	Single	None
Melting Curve Analysis (of the DNA-probe-hybrids)			
Programs/Cycle Program Data	***Value***		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	40	80
Hold/Incubation Time [h:min:s]	00:00:00	00:00:45	00:00:00
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	0.1
Sec Target/Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [cycles]	0	0	0
Acquisition Mode	None	None	Cont
Cooling (of the rotor and thermal chamber)			
Programs/Cycle Program Data	***Value***		
Cycles	1		
Analysis Mode	None		
Temperature Targets	***Segment 1***		
Target/Target Temperature [°C]	40		
Hold/Incubation Time [h:min:s]	00:00:30		
Ramp Rate/Temperature Transition Rate [°C/s]	20		
Sec Target/Secondary Target Temperature [°C]	0		
Step Size [°C]	0.0		
Step Delay [cycles]	0		
Acquisition Mode	None		

Fluorescence and Run Setup Parameters

Parameter	Setting
Seek Temperature	30 °C
Default channel • during run • for analysis	• Fluorescence channel 610 or 640 or 670 • refer to 2.3 Analysis
"Max. Seek Pos"	Enter the number of samples including controls.
"Instrument Type"	"6 Ch.": for LightCycler® 2.0 Instrument (selected by default)
"Capillary Size"	Select "20 µl" as the capillary size for the experiment.

Preparation of the PCR Mix

Proceed as described below to prepare a 20 µl standard reaction.
Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries in centrifuge adapters or in a LightCycler® Sample Carousel in a LC Carousel Centrifuge Bucket.
2. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
3. In a 1.5 ml reaction tube prepare the PCR mix by adding the following components in the order mentioned below, than mix gently by pipetting up and down.

The volumes indicated below are based on a single 20 µl standard reaction. 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
microproof Hygiene Screening System Master Mix (vial 1, yellow cap)	16.0 µl
microproof Enzyme Mix (Taq Polymerase / UNG) (not included)	0.5 µl[1]
microproof Hygiene Screening System Internal Control (vial 2, white cap)	1.0 µl
Total volume	17.5 µl

[1]if enzymes of other suppliers are used volumes may have to be adapted

4. • Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   • Pipet 17.5 µl PCR mix into each LightCycler® capillary.
   • For the samples of interest, add 2.5 µl sample DNA to a capillary, seal with a stopper.
   • For the negative control, add 2.5 µl H_2O PCR-grade (vial 4, colorless cap), seal with a stopper.
   • For the positive control, add 2.5 µl **micro**proof Hygiene Screening System Positive Control (vial 3, purple cap), seal with a stopper.
5. • 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 LC Carousel Centrifuge for spinning the capillaries.
6. Transfer the capillaries to the LightCycler®.
7. Cycle the samples as described above.

2.3 Analysis

Interpretation of Amplification Curves

Different fluorescence channels are used to monitor the amplification of DNA of isolates of the genus *Corynebacterium* and some coryneform bacteria (qualitative detection channel 610), isolates of the genera *Staphylococcus* and *Macrococcus* (qualitative detection channel 640), and isolates of the genera *Micrococcus, Kocuria*, and *Kytococcus*, and *Nesterenkonia halobia* (qualitative detection channel 670). The amplification signal of the Control Template can be detected in all three channels. The specific amplification of the Internal Control is analyzed in fluorescence 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, 610, 640, 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, and then 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, 610, 640, 670 and 705) then click OK.
5. The analysis charts are redrawn using the compensated data. Notice 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).

Compare the results from channel 610 (*Corynebacterium* spp., some coryneform bacteria), 640 (*Staphylococcus* spp., *Macrococcus* spp.), 670 (*Micrococcus* spp., *Kocuria* spp., *Kytococcus* spp., *Nesterenkonia halobia*), and channel 705 (Internal Control) for each sample, and interpret as described in the following table.

610	640	670	705	Result Interpretation
+	-	-	+ or -	positive for *Corynebacterium* spp. or for Coryneform Bacteria
-	+	-	+ or -	positive for *Staphylococcus* spp. or *Macrococcus* spp.
-	-	+	+ or -	positive for *Micrococcus* spp. or *Kocuria* spp. or *Kytococcus* spp. or *Nesterenkonia halobia*
-	-	-	+	negative for *Corynebacterium* spp. , *Staphylococcus* spp., *Macrococcus* spp., *Micrococcus* spp., *Kocuria* spp., *Kytococcus* spp., and *Nesterenkonia halobia*
-	-	-	-	invalid

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

A signal in more than one of the channels 610, 640, and 670 indicates either a contamination with Control Template DNA (Positive Control) or a mixture of organisms as sample material.

Interpretation of Melting Curves
A melting curve analysis (module Tm Analysis) can be performed in case of a positive result.

The following tables show the possible results and their interpretation:

Channel 610

Differentiation between the genus *Corynebacterium* and other coryneform bacteria:

Melting Peak Temperature	Result Interpretation
at least one peak/shoulder ≥ 63 °C	*Corynebacterium* sp.
all peaks < 63 °C	Coryneform bacterium

Note that only some coryneform bacteria are detected with the Hygiene Screening System. A negative result in channel 610 does not imply the absence of DNA of coryneform bacteria in general.

Channel 640

OPTIONAL/CONDITIONALLY: Differentiation between the closely related genera *Staphylococcus* and *Macrococcus*.

Note: It has been observed that the criteria for the differentiation of the two genera can vary between instruments and with different lots of reagents. The species of the genus *Macrococcus* are closely related to oxidase-positive *Staphylococcus* spp. and have been isolated from the skins of different mammals. Since they are quite uncommon they are very unlikely to occur in environmental monitoring samples.

Melting Peak Temperature	Result Interpretation
at least one peak/shoulder > 64 °C OR melting curve area at > 69 °C	*Staphylococcus* sp.
all peaks < 64 °C AND melting curve at baseline level at 69 °C	*Macrococcus* sp.

Channel 705

Identification of *Staphylococcus aureus* and *Micrococcus luteus*.

Note: Check the crossing points of the amplification curves in case the melting curve analysis in channel 705 yields no specific peak. It has been observed that for late amplification curves no further differentiation via melting curves may be possible. The respective limits for the crossing point values are given in the table. In order to see the crossing points (*i.e.* column “CP”) in the Qualitative Detection Analysis, you have to click the button “Advanced”.

Melting Peak Temperature	Result Interpretation	Crossing Point Limits
main peak at 70 °C (± 1.5 °C)	Internal Control	not applicable
peak at 58 °C (± 1.5 °C)	*Staphylococcus aureus*	< 22.5 in channel 640
peak at 60 °C (± 1.5 °C)	*Micrococcus luteus*[1] + Control Template	< 24.0 in channel 670

[1] a few isolates with an additional melting peak at approx. 53 °C have occurred

OPTIONAL/CONDITIONALLY: Identification of further groups/species.

The specific melting peak temperatures of *Staphylococcus aureus* and *Micrococcus luteus* in channel 705 have been validated with more than 50 strains of each species. The following tables give additional information on melting curves in channel 705 that have been observed but not thoroughly validated. Note that the crossing point limits given above for *S. aureus* and *M. luteus* apply as well.

Melting peak temperatures for *Staphylococcus* spp.:

Melting Peak Temperature (channel 705)	**Observed for Strains of ...**
42 .5 – 45.0 °C	*Staphylococcus arlettae*
47.5 – 50.5 °C	*S. capitis, S. caprae*[1], *S. epidermidis, S. haemolyticus*[1], *S. hominis*[2], *S. pasteuri, S. saccharolyticus, S. warneri*
51.0 – 53.5 °C	*Staphylococcus kloosii*

[1] a few isolates without the specific melting peak at approx. 49 °C have occurred
[2] one isolate with an additional melting peak at approx. 57 °C has occurred

Melting peak temperatures for organisms with a positive amplification signal in channel 670:

Melting Peak Temperature (channel 705)	**Observed for Strains of ...**
40.0 – 44.0 °C	*Kytococcus schroeteri, Kytococcus sedentarius, Nesterenkonia halobia* *Kocuria carniphila, Kocuria kristinae, Kocuria rhizophila, Kocuria varians,* and an as yet not classified *Kocuria* sp.
51.5 – 56.5 °C	*Kocuria palustris, Micrococcus flavus, Micrococcus lylae,* and two as yet not classified *Micrococcus* spp.

The peak height of positive samples may vary according to the initial cell concentration. In some cases the peaks above 63 °C in channels 610 and 640 may be reduced to a shoulder.

All shoulders/peaks > 62 °C in channel 705 belong to the Internal Control. Depending on the initial cell concentration, they may or may not be present in positive samples.

Note that the presence or absence of specific melting peaks should be checked manually for all positive samples as the peak finding algorithm of the LightCycler Software may not detect all relevant maxima of the melting curve. A guarantee for the identification via melting curves cannot be given.

A flow chart describing the analysis steps and possible result interpretations of unknown samples is provided as Annex 1 to this product insert.

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 610, 640, 640 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.
	Pre-Incubation step too short.	• Check the cycle programs. Some HotStart Taq polymerases need longer activation times.
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).
	Coryneform bacterium amplified but no amplification signal detected in channel 610.	• Check for melting curve in channel 610.
Fluorescence intensity is too low.	Inappropriate storage of components.	• Store the **micro**proof Hygiene Screening System Master Mix (vial 1, yellow cap) at -15 °C to –25 °C, protected from light. • Avoid repeated freezing and thawing.
	Hygiene Screening System Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the **micro**proof Hygiene Screening 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. • Use Uracil-DNA-Glycosylase (UNG) to inactivate amplificates from previous runs. UNG is included in Enzyme Mix (Taq Polymerase / UNG) from BIOTECON Diagnostics.
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.
S. aureus or *M. luteus* not identified (no melting curve in channel 705).	Too low initial amount of target DNA.	• Repeat the analysis with more colony material.

4. Additional Information on this Product

How this Product Works

The **micro**proof Hygiene Screening System provides primers and Hybridization Probes (for sequence-specific detection), convenient premixed reagents, and a control template 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 channels 610, 640, and 670. In case of a negative result due to inhibition ofamplification 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 Hygiene Screening System minimizes contamination risk and contains all reagents (except for Taq polymerase and template DNA) needed for detection of target DNA. 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.

Background Information

Fluorescence coupled PCR technology (polymerase chain reaction) is employed for the qualitative detection of major representative microorganisms found in the pharmaceutical environment (personnel, air, surface). For this purpose, a multiplex PCR system, the **micro**proof Hygiene Screening System has been developed by BIOTECON Diagnostics, which fast and reliably detects bacteria of the genus *Staphylococcus*, *Micrococcus,* and *Corynebacterium*. By use of different fluorescence channels, a stepwise assignment to either one or other of the genera is possible within a single PCR reaction. The system represents a new tool for the identification of environmental monitoring samples giving rise to results on the genus (and in some instances the species) level. Since 2004, the usage of more sensitive and specific tests is recommended by the European Pharmacopoeia (E.P., Chapters 5.1.6 and 2.6.1) and by the FDA Aseptic Guideline with reference to molecular biological methods. There is a need for new methods because most of the conventional methods are time-consuming and sometimes show an insufficient accuracy. A monitoring study in 2006 with several pharmaceutical companies revealed that up to 80% of the microorganisms isolated from samples derived from the pharmaceutical environment (personnel, air, surface) belong to the genera *Staphylococcus*, former genus *Micrococcus,* and *Corynebacterium*. The **micro**proof Hygiene Screening System allows the detection of all of these genera and the identification of the most important species, *Micrococcus luteus*, within less than 70 minutes. The remaining 20% of organisms found in the pharmaceutical environment can be further identified by conventional methods without any loss of time.

Quality Control

The **micro**proof Hygiene Screening System 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

License Notice

The purchase of this product includes a limited, non-transferable license under U.S. Patents Nos. 6,245,514 and 6,174,670, and corresponding patents and patent applications outside the United States, owned by the University of Utah Research Foundation and licensed by Idaho Technology, Inc. and Roche Diagnostics GmbH, to use only this amount of product for FRET assays solely for food analysis or GMO analysis. No right is conveyed, expressly, by implication or estoppel, for any other patent, such as under any patent for an apparatus or system, or to use this product for any other purpose.

5.3 Trademarks

microproof® is a trademark of BIOTECON Diagnostics GmbH.
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 2, October 2012
microproof® has been added as the new brand name for BIOTECON Diagnostics pharma related products.
Version 3, August 2014
The analysis schemes have been adapted due to new findings in the result interpretation.
Version 4, March 2017
License Notice changed.

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