

FOR *IN VITRO* USE ONLY

**Hygiene Screening System**  
**- Hybridization Probes -**  
**Manual**  
Version 07.09

Art. No. 943 101

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

**Store at -15 to -25 °C**

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### 1. What this Product Does

#### Number of Tests

The 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 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:

#### Contents

Vial / Cap Color	Label	Contents / Function / Storage
1 yellow cap	Hygiene Screening System Master Mix	<ul style="list-style-type: none"> <li>• 3 x 520 µl</li> <li>• Ready-to-use primer and Hybridization Probe mix for the specific amplification and detection of DNA of <i>Staphylococcus</i>, <i>Micrococcus</i>, <i>Corynebacterium</i>, and the Internal Control (IC).</li> <li>• Store at -15 to -25°C.</li> <li>• <b>Avoid repeated freezing and thawing!</b></li> <li>• <b>Protect from light!</b></li> </ul>
2 white cap	Hygiene Screening System Internal Control	<ul style="list-style-type: none"> <li>• 3 x 32 µl</li> <li>• Contains a stabilized solution of plasmid DNA.</li> <li>• For use as an internal amplification control.</li> <li>• Store at -15 to -25°C.</li> <li>• After first thawing store at +2 °C to +8 °C for up to one month.</li> </ul>
3 purple cap	Hygiene Screening System Positive Control	<ul style="list-style-type: none"> <li>• 1 x 50 µl</li> <li>• Contains a stabilized solution of plasmid DNA.</li> <li>• For use as a PCR run positive control.</li> <li>• Store at -15 to -25°C.</li> <li>• After first thawing store at +2 °C to +8 °C for up to one month.</li> </ul>
4 colorless cap	H <sub>2</sub> O, PCR-grade	<ul style="list-style-type: none"> <li>• 1 x 1 ml</li> <li>• Nuclease-free, PCR-grade H<sub>2</sub>O.</li> <li>• For use as a PCR run negative control.</li> <li>• Store at -15 to -25°C.</li> </ul>

**Additional Equipment and Reagents Required**

- LightCycler® Carousel-Based System (LightCycler® 2.0 Instrument, Roche Applied Science)<sup>2</sup>
- LightCycler® 20 µl - Capillaries<sup>2</sup>
- Hygiene Screening System Color Compensation Set<sup>1</sup>
- 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<sup>2</sup> for use with the LightCycler® 2.0 Sample Carousel (optional).
- Suspension Buffer<sup>1</sup>
- HotStart Taq polymerase, e.g. Enzyme Mix (Taq Polymerase/UNG)<sup>1</sup>  
(the 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

<sup>1</sup> Available from Biotest AG (Art. No. 943 103)

<sup>2</sup> Available from Roche Diagnostics

**Intended Use**

The Hygiene Screening System is designed for the rapid identification of microorganisms of the genera *Corynebacterium*, *Staphylococcus*, *Macroccoccus*, *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 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
<b>Total assay time</b>	<b>65 min</b>

## 2. How to Use this Product

### 2.1 Before You Begin

#### Precautions

Detection and identification of target DNA using the 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<sub>2</sub>O, 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 Enzyme Mix (Taq Polymerase / UNG) from Biotest AG. 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)			
<i>Programs/Cycle Program Data</i>		<i>Value</i>	
Cycles		35	
Analysis Mode		Quantification	
<i>Temperature Targets</i>		<i>Segment 1</i>	<i>Segment 2</i>
Target/Target Temperature [°C]		95	62
Hold/Incubation Time [h:min:s]		00:00:02	00:00:20
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	Single
Melting Curve Analysis (of the DNA-probe-hybrids)			
<i>Programs/Cycle Program Data</i>		<i>Value</i>	
Cycles		1	
Analysis Mode		Melting Curves	
<i>Temperature Targets</i>		<i>Segment 1</i>	<i>Segment 2</i>
Target/Target Temperature [°C]		95	40
Hold/Incubation Time [h:min:s]		00:00:00	00:00:45
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	Cont

Cooling (of the rotor and thermal chamber)	
<i>Programs/Cycle Program Data</i>	<i>Value</i>
Cycles	1
Analysis Mode	None
<i>Temperature Targets</i>	<i>Segment 1</i>
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	<ul style="list-style-type: none"> <li>• Fluorescence channel 610 or 640 or 670</li> <li>• refer to 2.3 Analysis</li> </ul>
"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, then 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
Hygiene Screening System Master Mix, (vial 1, yellow cap)	16.0 µl
Enzyme Mix (Taq Polymerase / UNG) (not included)	0.5 µl <sup>1</sup>
Hygiene Screening System Internal Control, (vial 2, white cap)	1.0 µl
<b>Total volume</b>	<b>17.5 µl</b>

<sup>1</sup> 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<sub>2</sub>O, PCR-grade (vial 4, colorless cap), seal with a stopper.
  - For the positive control, add 2.5 µl 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 *Micrococcus* (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 Positive Control 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, then select Select Color Compensation... .
2. Select the color compensation object you want to apply, 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., *Micrococcus* 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 <i>Corynebacterium</i> spp. or for Coryneform Bacteria
-	+	-	+ or -	positive for <i>Staphylococcus</i> spp. or <i>Micrococcus</i> spp.
-	-	+	+ or -	positive for <i>Micrococcus</i> spp. or <i>Kocuria</i> spp. or <i>Kytococcus</i> spp. or <i>Nesterenkonia halobia</i>
-	-	-	+	negative for <i>Corynebacterium</i> spp., <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>Micrococcus</i> spp., <i>Kocuria</i> spp., <i>Kytococcus</i> spp., and <i>Nesterenkonia halobia</i>
-	-	-	-	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 Positive Control DNA or a mixture of organisms as sample material.

### Interpretation of Melting Curves

A Melting Curve Analysis (module Tm Analysis) is performed in case of a positive result in order to

- differentiate between the closely related genera *Staphylococcus* and *Micrococcus*,
- differentiate between the genus *Corynebacterium* and other coryneform bacteria,
- identify *Staphylococcus aureus* and *Micrococcus luteus*.

The following table shows the possible results and their interpretation:

Detection Channel	Melting Peak Temperature	Result Interpretation
610	at least one peak/shoulder > 63°C	<i>Corynebacterium</i> sp.
	all peaks < 63°C	Coryneform bacterium <sup>1</sup>
640	at least one peak/shoulder > 64°C OR melting curve area at > 69°C	<i>Staphylococcus</i> sp.
	all peaks < 64°C AND melting curve at baseline level at 69°C	<i>Micrococcus</i> sp. <sup>2</sup>
705	main peak at 70°C (± 1.5°C)	Internal Control
	peak at 58°C (± 1.5°C)	<i>Staphylococcus aureus</i>
	peak at 60°C (± 1.5°C)	<i>Micrococcus luteus</i> Positive Control

<sup>1</sup> 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.

<sup>2</sup>The species of the genus *Micrococcus* 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 unlikely to occur in environmental monitoring samples.

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.

Melting peak temperatures for *Staphylococcus* spp.:

Melting Peak Temperature (channel 705)	Observed for Strains of ...
42.5 – 45.0°C	<i>Staphylococcus arlettae</i>
47.5 – 50.5°C	<i>S. capitis</i> , <i>S. caprae</i> <sup>1</sup> , <i>S. epidermidis</i> , <i>S. haemolyticus</i> <sup>1</sup> , <i>S. hominis</i> <sup>2</sup> , <i>S. pasteurii</i> , <i>S. saccharolyticus</i> , <i>S. warneri</i>
51.0 – 53.5°C	<i>Staphylococcus kloosii</i>

<sup>1</sup> a few isolates without the specific melting peak at approx. 49°C have occurred

<sup>2</sup> 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	<i>Kytococcus schroeteri</i> , <i>Kytococcus sedentarius</i> , <i>Nesterenkonia halobia</i> <i>Kocuria carniphila</i> , <i>Kocuria kristinae</i> , <i>Kocuria rhizophila</i> , <i>Kocuria varians</i> , and an as yet not classified <i>Kocuria</i> sp.
51.5 – 56.5°C	<i>Kocuria palustris</i> , <i>Micrococcus flavus</i> , <i>Micrococcus lylae</i> , and two as yet not classified <i>Micrococcus</i> 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.

### 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.	<ul style="list-style-type: none"> <li>• Check for correct pipetting scheme and reaction setup. Repeat the PCR run.</li> <li>• Always run a positive control along with your samples.</li> </ul>
	No data acquisition programmed.	<ul style="list-style-type: none"> <li>• Check the cycle programs.</li> <li>• Select acquisition mode "single" at the end of each annealing segment of the PCR program.</li> </ul>
	Pre-Incubation step too short.	<ul style="list-style-type: none"> <li>• Check the cycle programs. Some HotStart Taq polymerases need longer activation times.</li> </ul>
No signal increase in channel 705 is observed.	Inhibitory effects of the sample material (e.g., caused by too much cell material or DNA).	<ul style="list-style-type: none"> <li>• Use the recommended sample preparation.</li> <li>• Dilute sample extracts (e.g. in H<sub>2</sub>O, PCR-Grade, or Suspension Buffer).</li> </ul>
Fluorescence intensity is too low.	Inappropriate storage of components.	<ul style="list-style-type: none"> <li>• Store the Hygiene Screening System Master Mix (vial 1, yellow cap) at -15 °C to -25 °C, protected from light.</li> <li>• Avoid repeated freezing and thawing.</li> </ul>
	Hygiene Screening System Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the Hygiene Screening Master Mix (vial 1, yellow cap) thoroughly before pipetting.
	Too high initial amount of target DNA.	<ul style="list-style-type: none"> <li>• Dilute sample extracts (e.g. in H<sub>2</sub>O, PCR-Grade, or Suspension Buffer).</li> </ul>
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> <li>• Exchange all critical solutions.</li> <li>• Repeat the complete experiment with fresh aliquots of all reagents.</li> <li>• Always handle samples, reagents and consumables in accordance with commonly accepted practices to prevent carry-over contamination.</li> <li>• Add positive controls after sample capillaries and negative control capillaries have been sealed with stoppers.</li> <li>• Use Uracil-DNA-Glycosylase (UNG) to inactivate amplicates from previous runs. UNG is included in Enzyme Mix (Taq Polymerase / UNG) from <b>Biotest AG</b>.</li> </ul>
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 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 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 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, 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 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 Hygiene Screening System is function tested using the LightCycler® 2.0 Carousel-Based System.

## **5. Supplementary Information**

### **5.1 Ordering Information**

Biotest AG is offering a broad range of products for environmental monitoring. For a complete overview and for more information, please visit our website at [www.biotest.com](http://www.biotest.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. The product is covered in-part by US 5,871,908, co-exclusively licensed by Roche Diagnostics GmbH from Evotec OAI AG.

### **5.3 Trademarks**

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

### **5.4 Contact and Support**

For any inquiries or to place an order, contact your local Biotest distributor.

Domestic customers in Germany may contact Customer Service at:

Phone: +49 (0) 6103 801-496  
Fax: +49 (0) 6103 801-505  
Email: [mail@biotest.de](mailto:mail@biotest.de)

For technical or applicational inquiries please contact our Technical Support at:

Phone: +49 (0) 6221 72651-30  
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For other language versions of this manual, please see [www.biotest.de](http://www.biotest.de).

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