

# BactScreen ToolSet™ for LightCycler™

Lyophilised ToolSet for PCR using the LightCycler™ Instrument. Licensed by Roche Diagnostics GmbH

**Order#: BactScreen - 16**

1 ToolSet for 16 reactions

Store at 4°C, protected from light.  
Exposure to light may especially damage  
the OligoTool™ tube (vial with red cap).

For use with LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102)

## 1. ToolSet contents

Vial	Label	Content	Quantity
			<b>BactScreen - 16</b>
<b>1, Red cap</b>	<b>OligoTool</b>	- lyophilized oligos for PCR - contains mutation detection and anchor probe, primers	For 16 tests  Dissolved: 50 µL
<b>2 A-B, Green caps</b>	<b>Controls</b>	- lyophilized bacterial DNA of <b><i>E. coli</i></b> and <b><i>M. lysodeicticus</i></b> DNA	Dissolved: 20 µL each
<b>3, Blue cap</b>	<b>Solvent</b>	- to dissolve OligoTool / Controls	1000 µL of Solvent

Additional equipment and reagents required but not supplied :  
LightCycler-DNA Master Hybridization Probes, 10 x conc. Cat.No.: 2 015 102, including 25mM MgCl<sub>2</sub>;  
LightCycler instrument, LightCycler capillaries, DNA extraction materials

## 2. Introduction

### 2.1. Product overview

#### ToolSet description

The **BactScreen ToolSet** is specifically adapted for amplification of eubacterial 16S rRNA-DNA and distinction of Gram-Negative and Gram-Positive species by LightCycler PCR with Melting Curve Analysis. Fluorescent detection and anchor probes and the primer pair have been optimized for specific amplification of targets and **Gram class assignment by SNP discrimination**.

Purified PCR products may be sequenced if desired (for primers see page 2).

#### Control material

**Control A** = *E. coli* DNA; **Control B** = *M. lysodeicticus* DNA; lyophilized.

#### Storage of ToolSet and Solutions

Store at +4°C when lyophilized, protected from light.  
The unopened lyophilized ToolSet is stable at +4°C for 12 months from date of manufacture if protected from light. When dissolved store at +4°C for a maximum of 2 weeks, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing more than once.

### 3. Preparation for LightCycler PCR

**Toolset preparation** Dissolve the content of the OligoTool tube (Red Cap) with 50 µl of Solvent.  
Dissolve the content of the Control tubes (Green Caps) with 20 µl of Solvent.

1. Before opening tubes, centrifuge them quickly.
2. Add Solvent into OligoTool tube and Control tube as above.
3. Recap tubes, vortex gently.
4. Before opening tubes, centrifuge them quickly.
5. Proceed to Reaction Mix preparation.

**Primers ?** You don't have to add primers.  
**Probes ?** You don't have to add probes.

**Reaction Mix Preparation** For 1 (One) reaction, prepare the Reaction Mix as shown in the following table :

Reagent	µL
OligoTool <b>BactScreen</b> -16 dissolved	2.8
<b>PCR grade Water</b>	9.6
MgCl <sub>2</sub> 25 mM	1.6
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control <b>BactScreen</b> -16	4
Grand Total	20

Use Master Hybridization Probes 10x, PCR grade Water and MgCl<sub>2</sub> 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, including PCR grade Water and 25mM MgCl<sub>2</sub>). For multiple reactions, multiply the indicated volumes appropriately.

**Positive Control** Always run a positive control with the samples. Use the dissolved BactScreen Controls A and B (*E. coli* or *M. lysodeicticus* DNA, both Green Caps).

**Negative control** Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Solvent (Blue Cap).

**Extraction of bacterial DNA** Due to variable source materials, no universal method is applicable. Follow instructions of manufacturers or use published protocols. Final buffers should be salt-free or low salt.

**Application** The **BactScreen** ToolSet™ for LightCycler™ allows **detection of most Eubacteria** with a sensitivity of approximately 10-100 CFU depending on starting material and conditions **and T<sub>m</sub> – based Gram-typing**. The following bacteria were correctly grouped by Melting curve analysis using the BactScreen ToolSet :

**Gram - Negative Group 1 (GN1)** : *Escherichia coli*, *Citrobacter freundii*, *Haemophilus influenzae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Pasteurella aerogenes*

**Gram - Negative Group 2 (GN 2)** : *Brucella melitensis*

**Gram - Negative Group 3 (GN 3)** : *Bacteroides fragilis*

**Gram - Positive Group (GP)** : *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Micrococcus lysodeicticus*

**Note** : See page 4 for details and Appendix for an extended list of bacteria.

Note : This ToolSet was developed for use in life science research only.

**Sequencing** For sequencing of amplicons, use the following primers (not provided) :

BAC 5' = 5'-TTAgATACCCTggTAgTCCAC-3'

BAS 3' = 5'-CTgATCYRCgATTACTAgCgA-3'

## 4. LightCycler Settings and Experimental Protocol

### Denaturation

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	<b>Segment 1</b>
Target Temperature (°C)	95
Incubation time (s)	60
Temperature Transition Rate (°/s)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

### Amplification

Cycle Program Data	Value		
Cycles	40 - 50		
Analysis Mode	None		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	48	72
Incubation time (s)	0	20	20
Temperature Transition Rate (°/s)	20.0	20.0	20.0
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	Single	None

**NOTE** : At > 40-50 cycles the negative control may amplify due to bacterial DNA traces in polymerase etc.

### Melting Curve Analysis

Cycle Program Data	Value		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	35	70
Incubation time (s)	10	30	0
Temperature Transition Rate (°/s)	20.0	20.0	0.1 (0.05 HiRes)
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	None	Continuous

### Cooling

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	<b>Segment 1</b>
Target Temperature (°C)	40
Incubation time (s)	30
Temperature Transition Rate (°/s)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

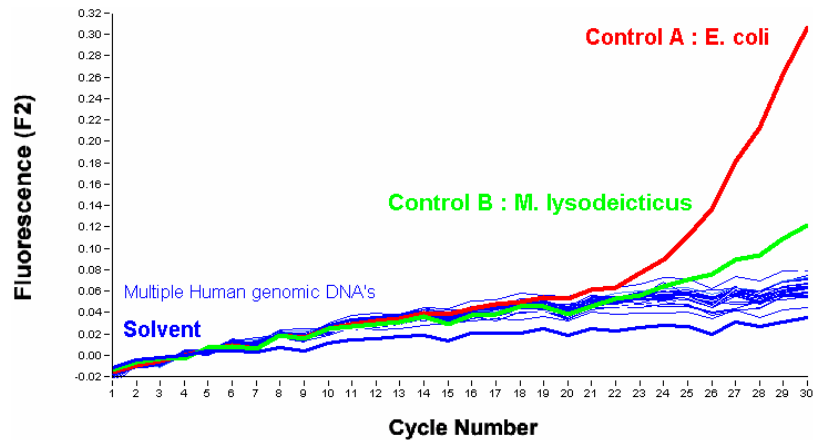
### Fluorescence display mode

Use F2/F1 or preferably F2 with colour compensation and automatic gain setting with LC program 3.5.  
Use gains: F1=1; F2=15 with earlier LC program versions.

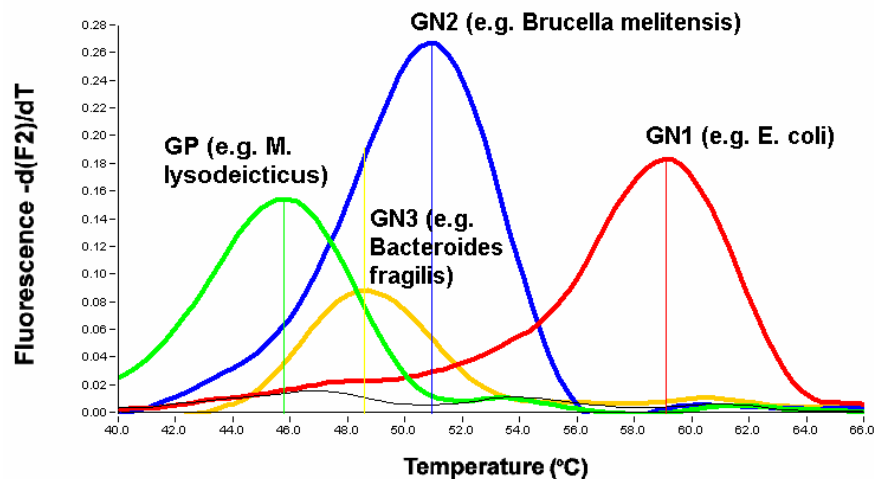
## 5. Typical results

### Introduction

Use the **Quantitation** program to analyse amplification of bacterial DNA. **Figure 1** shows a typical **Quantitation** obtained with the **BactScreen ToolSet™** for LightCycler™ :



Use the **Melting Curve** program to genotype the amplicons. **Figure 2** shows a typical **Melting Curve** analysis with the **BactScreen ToolSet™** for LightCycler™ :



**RED** : Control A = *E. coli* DNA, **GREEN** : Control B = *M. lysodeicticus* DNA ; both contained in the ToolSet.  
**BLUE** : *Brucella melitensis* DNA, **YELLOW** : *Bacteroides fragilis* DNA, **BLACK** : No DNA or human DNA.  
Conditions : LC program 3.5, Color compensation and Digital Filter enabled, Degrees to average : 10.0,  
Calculation Method : Polynomial. Green  $T_m = 45.7^\circ\text{C}$ , Yellow  $T_m = 48.6^\circ\text{C}$ , Blue  $T_m = 50.9^\circ\text{C}$ , Red  $T_m = 59.1^\circ\text{C}$ .

**Note** : The values for the respective melting temperatures may vary for  $\pm 2.5^\circ\text{C}$  between different experiments. The Delta T between the melting peaks for different genotypes may vary  $\pm 0.5^\circ\text{C}$ . The BactScreen ToolSet™ has been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Performance of the ToolSet with other instruments, accessories and reagents has not been validated by Genes-4U.

### 7. Notices to Purchaser : Licenses and Trademarks, Prohibition of Resale

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### How to contact Genes-4U

E-mail  
Internet

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**BactScreen Appendix Legend :** **Model Organism.** Tested organism. *GenBank comparison only.*

**Gram - Negative Group 1 = GN1,  $T_m \sim 59^\circ\text{C}$ ,** probe complementary to organism sequence :

*Aeromonas caviae*  
*Aeromonas hydrophila*  
*Acinetobacter baumannii*  
*Citrobacter freundii*  
**Escherichia coli**  
*Enterobacter aerogenes*  
*Helicobacter pylori*  
*Haemophilus influenzae*  
*Klebsiella pneumoniae*  
*Moraxella (Branhamella) catarrhalis*  
*Pasteurella aerogenes*  
*Proteus mirabilis*  
*Pseudomonas aeruginosa*  
*Salmonella typhi*  
*Serratia marcescens*  
*Stenotrophomonas maltophilia*

**Gram - Negative Group 2 = GN2,  $T_m \sim 51^\circ\text{C}$  or  $8^\circ\text{C}$  lower than GN1,** 1 mismatch with probe :

*Brucella melitensis*  
*Brucella abortus*  
*Campylobacter jejuni*  
*Campylobacter species*  
*Gardnerella vaginalis*

**Gram - Negative Group 3 = GN3,  $T_m \sim 48^\circ\text{C}$  or  $11^\circ\text{C}$  lower than GN1,** 2 minor mismatches with probe :

*Bacteroides fragilis*  
*Prevotella species*

**Gram - Positive Group = GP,  $T_m \sim 46^\circ\text{C}$ ,** or  $13^\circ\text{C}$  lower than GN1, 2 major mismatches with probe :

*Actinomyces species orale*  
*Bacillus subtilis*  
*Clostridium difficile*  
*Clostridium perfringens*  
*Corynebacterium diphtheriae*  
*Enterococcus faecalis*  
**Micrococcus lysodeicticus**  
*Mycobacterium tuberculosis*  
*Nocardia asteroides*  
*Peptostreptococcus species*  
*Propionibacterium species*  
*Staphylococcus aureus*  
*Staphylococcus epidermidis*  
*Streptococcus mitis*  
*Streptococcus pneumoniae*  
*Streptococcus pyogenes*  
*Veillonella species*

**Note :** Due to further variations in 16S rRNA-DNA sequence, bacteria other than those listed may exhibit melting curves that deviate characteristically in  $T_m$  from the patterns indicated.

**Genes-4U will calculate expected  $T_m$  for other bacteria. Address requests to : [Info@Genes-4U.com](mailto:Info@Genes-4U.com)**