

# BactoTyping Kit GTB *S. aureus*

Order No.: DVET004

**Unit: 100 strain typing  
200 reactions**

2 x 50 reactions *lucE* (FAM-None) green cap  
2 x 50 reactions *sea* (FAM-None) and *sed* (VIC-None) purple cap

For veterinary use only

## 1. Contents

Labelling	Content	Amount	Storage
GTB – <i>S. aureus lucE</i> (green cap)	Primer and probe (FAM) for <i>lucE</i> -detection	2 x 50 µl	-20°C
GTB – <i>S. aureus sea &amp; sed</i> (purple cap)	Primer and probe (FAM) for <i>sea</i> -detection, Primer and probe (VIC) for <i>sed</i> -detection	2 x 50 µl	-20°C
DNA Reaction Mix (white cap) <sup>#</sup>	Reaction Mix	4 x 500 µl	-20°C until first use, then at +4°C
<i>Staphylococcus aureus</i> Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	-20°C

<sup>#</sup>DNA Reaction Mix contains uracil-N glycosylase (UNG)

## 2. PCR setup

Set up two independent reactions for *lucE*, *sea* & *sed*

### *lucE*

		Per sample
<b>Preparation of master mix</b> (mix well)	H <sub>2</sub> O DNA Reaction Mix GTB- <i>Staphylococcus aureus lucE</i>	4.0 µl 10.0 µl 1.0 µl
	<b>Total volume</b>	<b>15.0 µl</b>
<b>Preparation of PCR assay</b>	Master mix Sample	15.0 µl 5.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

### *sea & sed*

		Per sample
<b>Preparation of master mix</b> (mix well)	H <sub>2</sub> O DNA Reaction Mix GTB- <i>Staphylococcus aureus sea &amp; sed</i>	4.0 µl 10.0 µl 1.0 µl
	<b>Total volume</b>	<b>15.0 µl</b>
<b>Preparation of PCR assay</b>	Master mix Sample	15.0 µl 5.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

### 3. Programming of the temperature profile

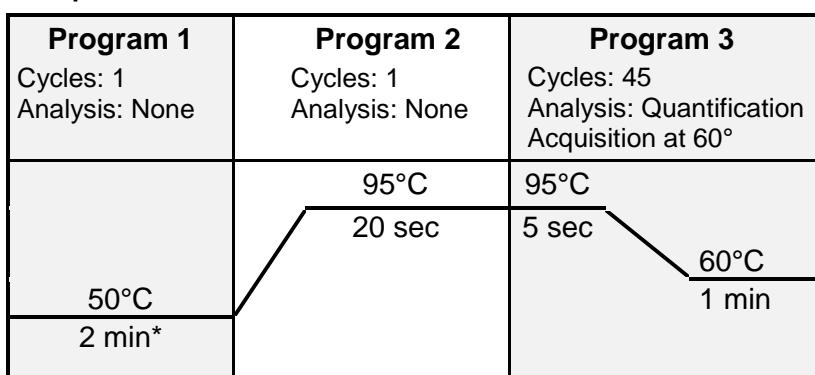
Please find further information on programming the real-time PCR instrument in the respective operator's manual. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

- Select dyes:**
- For detection of GTB *Staphylococcus aureus* *lucE*: FAM-NONE
  - For detection of GTB *Staphylococcus aureus* *sea*: FAM-NONE
  - For detection of GTB *Staphylococcus aureus* *sed*: VIC-NONE

**Select reference dye (passive reference):** ROX

**Sample Volume:** 20 µl

**Temperature Profile:**



For ABI PRISM® 7500:

Ramp speed: Without "fast cycling" parameter

For LightCycler® 480 instrument:

Detection format: 2 Color Hydrolysis Probe  
(dyes see above)

**\*Note:** If viral RNA should be also detected in the same PCR run, program 1 has to be prolonged to 15 min at 50°C. This temperature profile can be used for all BactoReal®, MycoReal, ParoReal and ViroReal® kits for the detection of DNA or RNA.

### 4. Results

***Staphylococcus aureus* genotype B:**

- *lucE* positive in most cases (point-mutation at nucleotide 132 of the *lucE* gene)
- *sea* frequently positive
- *sed* frequently positive

***Staphylococcus aureus* genotype C:**

- all negative

**Others:**

- *lucE* could be positive in some cases
- *sea* should be negative

**Lit.:** C. Fournier, P. Kuhnert, J. Frey, R. Miserez, M. Kirchhofer, T. Kaufmann, A. Steiner, H.U. Gruber: Bovine *Staphylococcus aureus*: association of virulence genes, genotypes and clinical outcome, Res. Vet. Sci., 85 (2008), pp. 439-448