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2-Color *Staphylococcus aureus* Detection Kit

Probe based detection kit for *Staphylococcus auerus*
SAMPLE KIT

RESEARCH USE ONLY

Contents

Product Description

The 2-color *Staphylococcus aureus* detection kit is a probe based kit that allows for the identification of *Staphylococcus aureus* strains. The primers and probes target a *Staphylococcus aureus* specific gene that encodes the thermos-stable nuclease essential for its pathogenicity. The probe activates FAM channel, giving the user a positive result if *Staphylococcus aureus* is present. An internal control primer and probes are also included that ensure that the PCR Master Mix is working optimally.

The **2X *Staphylococcus aureus* Detection Master Mix** has been tested for up to 20 freeze thaw cycles without a significant loss of activity.

Included in the Kit

- Qty. 1 – 2X *Staphylococcus aureus* Detection Master Mix (240 µL, 25 rxn total)
- Qty. 1 – *Staphylococcus aureus* Positive Control Template (FAM) (20 µL, 10 rxn)
- Qty. 1 – Internal Control Template (HEX/VIC) (50 µL, 25 rxn)
- Qty. 1 – 20X Yellow Dye (1.0 mL)
- Qty. 1 – ROX (0.1 mL)
- Qty. 2 – Water (RNase/DNase/Protease-free) (1.0 mL/tube)

Required But Not Provided

Equipment/Disposables

- qPCR machine and compatible plate(s) or strip tube(s)
- Bench-top centrifuge
- Aerosol filter pipette tips
- Pipettes
- Disposable nitrile gloves
- 1.5 mL microcentrifuge tubes

Storage

- Store all components at –20 °C

Before First Use

qPCR instruments will generally be classified as being compatible with either a ‘high’, ‘low’ or ‘no’ level of ROX. To get meaningful results, it is therefore critical to ensure that the correct amount of ROX is added to the **2X *Staphylococcus aureus* Detection Master Mix** before first use. List of instruments and their ROX levels is provided on page 6 of this manual. *If your machine is not listed and you do not know the ROX compatibility of your instrument, please consult your instrument manual*.

To reconstitute the Master Mix with the correct level of ROX:

1. Thaw both tubes of **2X *Staphylococcus aureus* Detection Master Mix** on ice.
2. Thaw **ROX** at room temperature (protect from direct light).
3. Depending on the ROX sensitivity of the instrument, add the following amount of **ROX** and water into each tube of **2X *Staphylococcus aureus* Detection Master Mix**:

Instrument ROX Requirement	ROX (µL)	Water (µL)
High Rox	10	0
Low ROX	9.9	0.1
No ROX	0	10

4. Mix all components in each **2X *Staphylococcus aureus* Detection Master Mix** by pipetting up and down 10 times.

The **2X *Staphylococcus aureus* Detection Master Mix** is ready to use.

Protocol

Note: The 2-color *Staphylococcus aureus* detection qPCR kit is capable of amplifying < 100 copies of *Staphylococcus aureus* DNA. Care must therefore be taken to avoid contamination of the kit components with *Staphylococcus aureus* DNA template. It is highly recommended to only use aerosol resistant filtered pipette tips under laminar flow during reaction setup. If contamination is an issue, clean the pipettes and lab bench with 3–6% hypochlorite and use a recently autoclaved or new set of aerosol resistant filtered pipette tips for reaction setup.

Note: Ensure all components of the kit are thawed and kept at 4 °C or on ice during reaction setup.

Workflow

Important: *Make sure the **2X Staphylococcus aureus Detection Master Mix** has been reconstituted with the correct amount of ROX ("Before First Use", page 3).*

1. Thaw all components on ice. It is necessary to keep all components on ice during reaction setup.
2. Invert each tube 10 times to mix and briefly centrifuge to collect liquid at the bottom of the tube. For tubes containing a low volume of liquid, you may flick the side of the tube 2–3 times, then briefly centrifuge to collect liquid at the bottom of the tube.
3. Add 10 µL of **2X *Staphylococcus aureus* Detection Master Mix** to a well of a qPCR plate.

Note: qPCR plate with the **2X *Staphylococcus aureus* Detection Master Mix** can be kept at room temperature while preparing components in step 4. If a delay is to be expected before proceeding to or during step 4 (up to 10 minutes), the qPCR plate should be placed at 4 °C.

4. For every test sample, positive and negative control, mix the following components in a 1.5 mL microcentrifuge tube (if performing experiments on technical replicates, adjust volumes accordingly):

Component	Volume (10 µL)
20X Yellow Dye	1 µL
DNA Template*	1–6 µL
Internal Control Template	2 µL
Water	up to 10 µL

* For positive control, add 2 µL of *Staphylococcus aureus* positive control template instead of your DNA template. For negative control (no template), do not add any DNA.

5. Ensure components in tube are mixed thoroughly by pipetting or brief vortexing.
6. Briefly centrifuge to collect liquid at the bottom of the tube.
7. Pipette the reaction mixture (10 µL) into wells of a qPCR compatible plate or strip tube containing the **2X *Staphylococcus aureus* Detection Master Mix**. The color of the solution should turn green.
8. Briefly centrifuge to collect liquid at the bottom of the well/strip tube(s).
9. Perform PCR using the following recommended guidelines:

Step	Temperature (°C)	Time	Number of Cycles
Denature	95	2 min	1
qPCR Detection	95	10 sec	35
	60	30 sec	

Interpretation of Results

	Internal Control Ct	Negative Control Ct	Sample Ct	*Staphylococcus aureus* Status of Sample
Scenario 1	< 25	–	< 30	POSITIVE
Scenario 2	< 25	–	–	NEGATIVE
Scenario 3	–	–	< 30	POSITIVE
Scenario 4	–	–	–	TEST INVALID

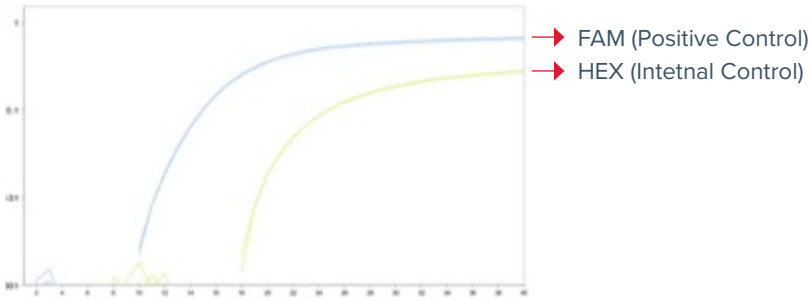
Scenario 1: Amplification occurred in the well with sample (negative control is blank). *Conclusion*: *Staphylococcus aureus* DNA detected in sample. *Note*: In cases where a Ct value is observed in the negative control well, the result may still be considered 'positive' if the Ct value of the sample is at least 3 Ct's lower than the negative control.

Scenario 2: Amplification is observed only in the internal control sample. *Conclusion*: *Staphylococcus aureus* DNA not detected in sample.

Scenario 3: Same as scenario 1 except no internal control amplification is observed. *Conclusion*: This still constitutes a positive result. The internal control is only informative of a failed qPCR run ***if*** the sample does not amplify.

Scenario 4: If no signal or Ct is observed in the internal control or the sample, the qPCR run is considered unsuccessful. The test is invalid and must be repeated.

Expected Results



Internal Control Ct	Positive Control Ct	No Template or Negative Control Ct
< 20	< 20	Undetermined or ≥ 34

Table 1. Summary of expected results when using the 2-color *Staphylococcus aureus* kit with the internal and positive controls included in the kit.

qPCR Instrument ROX Compatibility Chart

The chart below provides a list of qPCR machines, their manufacturer and ROX compatibilty.

ROX Content	Provider	Real Time PCR Instrument
No ROX (i.e. ROX not recommended)	Bio-Rad	iQ™5, CFX96, CFX384
	Roche	Opticon Lightcycler
	Qiagen	Rotor-Gene™
	Eppendorf	Mastercycler
	Cepheid	SmartCycler
	Antylia Scientific	Eco 48
Low ROX	Bio-Rad	iCycler, MyiQ, MiQ 2, iQ 5, CFX96, CFX384, Chromo4, MJOpticon, Opticon 2, MiniOpticon
	Cepheid	SmartCycler
	Eppendorf	Mastercycler
	Illumina	Eco Real-Time qPCR System
	Qiagen	Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000
	Roche	LightCycler 480, LightCycler 2.0
	Stratagene	MXP4000P, MX3000P, MX3005P
	ABI	7500, 7500Fast, ViiA 7, QuantStudio™ 3, QuantStudio™ 5, QuantStudio™ 6, QuantStudio™ 7, QuantStudio™ 12K, Flex
ROX or High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HTFast, StepOne, StepOnePlus

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