

## Master mix kits for TaqMan® probes

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*Master mix kits for TaqMan® probes are a convenient method for achieving successful results without optimizing reaction conditions.*

### Introduction

TaqMan probes are oligonucleotides that contain a reporter fluorophore at one end and a quencher at the other end. The excitation spectrum of the quencher overlaps the emission spectra of the fluorophore, thus quenching it (Ref 1, 2). During the course of PCR amplification, the 5'→3' exonuclease activity of the DNA polymerase degrades the annealed probe and releases the fluorophore into solution. This results in an increase in fluorescent signal (Ref 1).

For real-time PCR, the advantages of TaqMan probes include greater detection specificity than DNA intercalating dyes such as SYBR® Green I (Ref 3, 4) and the ability to perform genotyping of single nucleotide polymorphisms (SNPs) (Ref 5, 6).

Recognizing the popularity of TaqMan probes, various manufacturers have developed master mix kits that aim to improve convenience and standardization of reagents and reduce the need to optimize reaction conditions. With these kits, the user simply combines the master mix solution with template DNA, primers and probes and then runs the reaction without any optimization.

In this study, we compare 5 commercially available master mix kits for their use with TaqMan probes on the Spartan DX™ personal DNA analyzer.

### Materials and Methods

#### DNA extraction

DNA was isolated from clinical isolates of *Staphylococcus aureus*. For each isolate, 4-5 medium-sized bacterial colonies were re-suspended in 100 µl of lysis buffer (50 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, pH 8) with 2 µl of 1 mg/ml lysostaphin (Sigma-Aldrich, Cat No. I7386). The samples were incubated at 37°C for 30 min. Following this incubation, 5 µl of 20 mg/ml Proteinase K (Sigma-Aldrich, Cat. No. p2308) was added to the mixture and the tubes were shaken at 50°C for 1 h. The tubes were then incubated at 100°C for 10 min to inactivate the Proteinase K. Samples at a concentration of 750 ng/µl were stored at 4°C.

#### Real-time PCR

Oligonucleotide primers were designed against the Sa442

gene, which is specific to *Staphylococcus aureus*. The forward primer sequence was 5'-tcg gta cac gat att ctt cac-3', and the reverse primer was 5'-act ctc gta tga cca gct tc-3'. The expected amplicon size was 179 bp (Ref 7).

TaqMan probes were obtained from Biosearch Technologies. The probe contained a FAM fluorophore on the 5' end with a BHQ-1 quencher on the 3' end. The probe sequence was 5'-tac tga aat ctc att acg ttg cat cgg aaa ca-3'.

The following master mix kits were tested: QuantiMix Easy Probes (Biotools, Cat. No. 10.601), TaqMan® Fast Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems, Cat. No. 4352042), JumpStart™ Taq ReadyMix™ for Quantitative PCR (Sigma-Aldrich, Cat. No. D7440), Platinum® Quantitative PCR SuperMix-UDG (Invitrogen, Cat. No. 11730-017), iQ Supermix (Bio-Rad, Cat. No. 170-8860), TaqMan® Genotyping Master Mix (Applied Biosystems, Cat. No. 4371353) and FastStart TaqMan® Probe Master (Roche, Cat. No. 4673450001). A homebrew mix (Ref 8) was also tested using the components in Table 1 together with final concentrations of 1X PCR Reaction Buffer (no MgCl<sub>2</sub>) (Invitrogen), 2.5 mM MgCl<sub>2</sub> (Invitrogen), 0.125 mM dNTP mix (Invitrogen, Cat. No. 10297018), and 1.5 U Taq DNA polymerase (Biotools Cat. No. BT10048).

TaqMan reaction mixtures were assembled according to the manufacturers' instructions. Common components of the reaction mixtures are listed in Table 1. The same primer and probe concentrations were used for all kits. MgCl<sub>2</sub> concentration was adjusted to 2.5 mM, where applicable. Cycling parameters are listed in Table 2. For kits recommending an initial hot start, the mixes were pre-incubated at 95.0°C for the times indicated in Table 3. Reactions were performed on the Spartan DX™ using 0.2 ml thin-wall flat cap PCR tubes (Axygen, Cat. No. PCR-02C), and topped with 15 µl of mineral

Component	Final Concentration
Sa442 primers	0.5 µM each
Sa442 TaqMan probe	0.1 µM
Template DNA	1.5 µg
<b>Total reaction volume</b>	<b>20 µl</b>

**Table 1.** Common components of amplification mixtures.

Step	Temperature	Time	Cycles
Initial denaturation	92.5°C	4 s	1
Denaturation	92.5°C	45 s	35
Annealing/extension	52.4°C	45 s	35

**Table 2.** Cycling parameters.

oil (Biotools, Cat. No. 20.032) to prevent evaporation..

#### DNA analysis

Fluorescence values were downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 10 µl of the amplification products.

#### Results

Threshold cycle (Ct) values for the master mix kits varied between 13 and 18 cycles (Table 3). Most of the kits required an initial hot start step, which increased run times. The homebrew reaction mix performed comparably to the commercial kits. There was no observable difference in specificity of results between Hot-Start and non-Hot-Start kits.

#### Discussion and Conclusions

All of the master mix kits worked with TaqMan probes on the Spartan DX. Master mix kits with hot-start enzymes had longer run times because of the longer initial Hot-Start time and generated equivalent performance to non-Hot-Start

#### References

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#### Disclaimer

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Master mix kit	Hot Start	Ct
Homebrew mix	No	16.0 ± 0.0
QuantiMix Easy Probes (Biotools)	No	13.3 ± 0.6
TaqMan® Fast Universal PCR Master Mix (Applied Biosystems)	No	16.7 ± 1.0
JumpStart™ Taq ReadyMix™ for Quantitative PCR (Sigma-Aldrich)	1 min	15.7 ± 0.6
Platinum® Quantitative PCR SuperMix-UDG (Invitrogen)	2 min	15.3 ± 0.6
iQ Supermix (Bio-Rad)	3 min	15.0 ± 0.6
TaqMan® Genotyping Master Mix (Applied Biosystems)	10 min	16.6 ± 0.6
FastStart TaqMan® Probe Master (Roche)	10 min	18.0 ± 2.1

**Table 3.** Master mix kits and threshold cycles (Ct).

kits and a homebrew mix. If faster run times are important, we recommend using master mix kits with shorter hot-start times. In summary, master mix kits from a variety of manufacturers generated successful TaqMan results with no optimization of reaction conditions.