

## Fluorophores Compatible with the Spartan DX-12®

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The Spartan DX-12® is compatible with green- orange- and red-emitting fluorophores. Compatible fluorophores include FAM, CAL Fluor® Orange 560, CAL Fluor® Red 590, CAL Fluor® Red 610 and CAL Fluor® Red 635.

### Introduction

There are a variety of commercially-available Real-Time PCR chemistries such as TaqMan® (Applied Biosystems), Molecular Beacons (PHRI), and Scorpions® (DxS). The Spartan DX-12® instrument may be used with any of these chemistries, provided that compatible fluorophores are used for detection. The optical detection system in the Spartan DX-12® is able to detect fluorophores that excite at wavelengths between 471-492 nm and 555-584 nm, and emit at wavelengths between 520-532 nm and 620-800 nm.

The purpose of this study was to evaluate the compatibility of different fluorophores for the Spartan DX-12®.

### Materials and Methods

#### DNA preparation

DNA was isolated from buccal cells using the BuccalQuick Kit

(TrimGen, Cat. No. BQ-50), according to the manufacturer's instructions. The samples were incubated at room temperature for 5 min in BQ-Solution followed by the addition of 50 µl of BQ-Mix. Samples were stored at -20°C. The DNA had a concentration of 50 ng/µL.

#### Real-Time PCR

Primers were designed against CHRM3, a highly conserved region of the human muscarinic acetylcholine receptor subtype M3 gene. Eight (8) TaqMan® fluorophore-quencher probe combinations were used. The probe sequence was the same for all. The primer and probe sequences are shown in Table 1 and the fluorophores may be found in Table 2. Components of the Real-Time PCR mixture are listed in Table 3.

Samples were amplified in Spartan Tubes (Tubes, Cat. No.01004153 and Caps, Cat.No.01004155), and amplification was performed in triplicate on Spartan DX-12® instruments. Table 4 shows the cycling parameters.

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
CHRM3 primers	TTg ggT CAT CTC CTT Tg	gCA CAg TTC TCT TTC CA	74
CHRM3 probe	Fl - TCC TTT ggg CTC CTg CCA TCT - Q		

Fl = fluorophore, Q = Quencher

**Table 1.** Primer/probe sequences and amplicon sizes.

Fluorophore	Excitation (nm)	Emission (nm)
FAM	494	522
CAL Fluor® Orange 560	538	559
Quasar® 570	548	566
CAL Fluor® Red 590	569	591
CAL Fluor® Red 610	590	610
CAL Fluor® Red 635	616	637
Pulsar® 650	460	650
Quasar® 670	644	670
Quasar® 705	690	705

**Table 2.** Fluorophore excitation and emission wavelengths.

Component	Final amount
10X PCR Reaction Buffer (No MgCl <sub>2</sub> ) (Invitrogen)	1 X
MgCl <sub>2</sub> (Invitrogen)	5.75 mM
dNTP mix (Biotools)	0.2 mM
Taq DNA polymerase (Biotools)	1 U
TaqMan® Probes (Biosearch Technologies)	0.5 X
PCR primers (Biosearch Technologies)	0.3 µM/each*
Template DNA	1 µl
Sterile water	
<b>Total reaction volume</b>	<b>20 µl</b>

\*Unless otherwise indicated.

**Table 3.** Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	150 s	1
Denaturation	95°C	30 s	40
Annealing/extension	60°C	55 s	40

**Table 4.** Cycling parameters.

### DNA analysis

Fluorescence data was transferred from the Spartan DX-12® and graphed using the Spartan Graphing & Analysis Software (ver. 3.10). PCR results were verified using a 2% agarose gel electrophoresis with 10 µL of the amplification products.

### Results

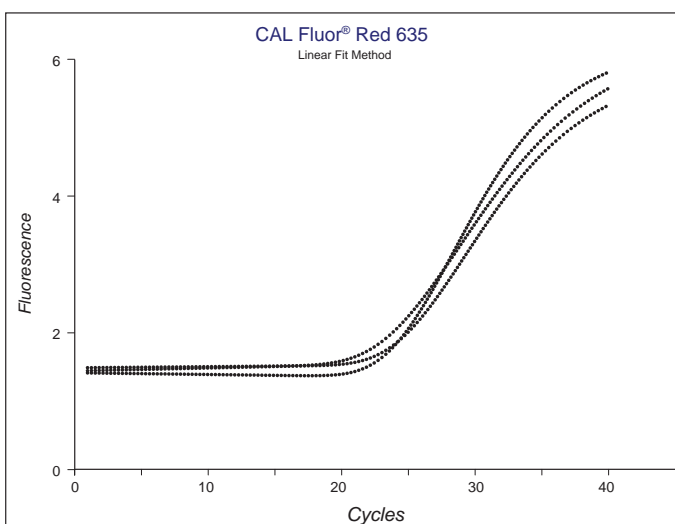
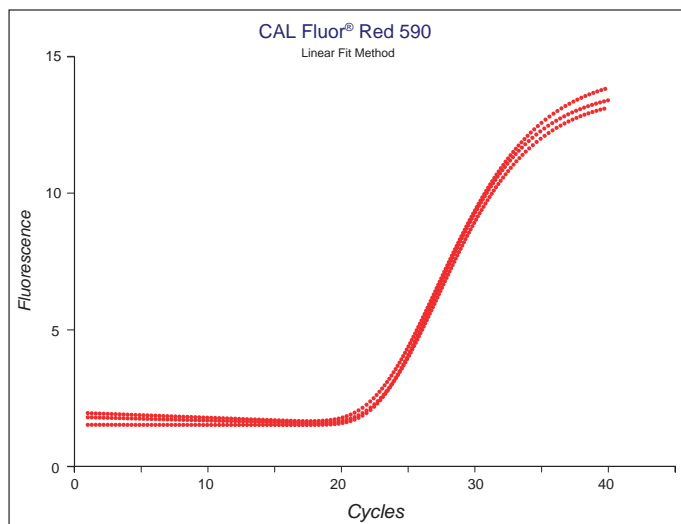
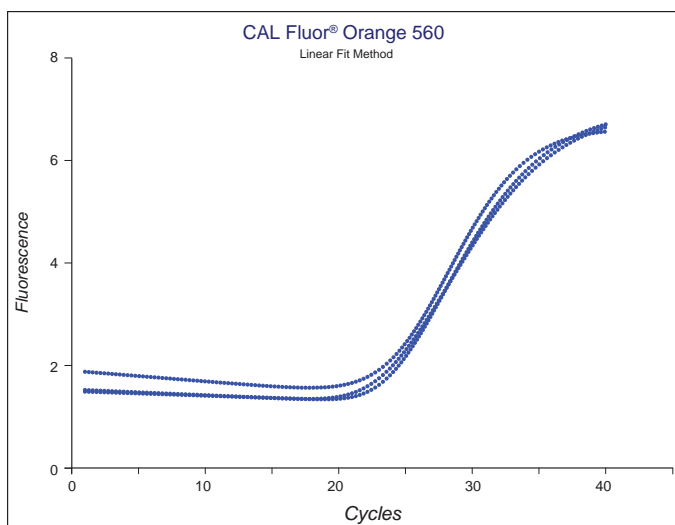
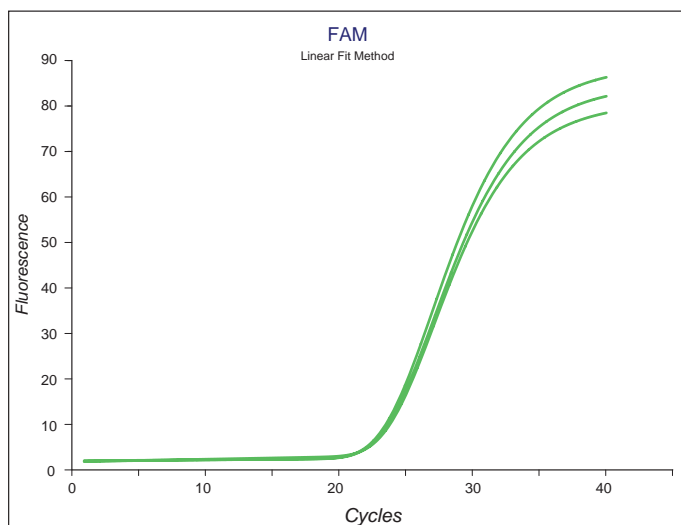
Gel electrophoresis revealed that all reactions yielded a single amplicon of the correct size. Real-Time PCR amplification plots showed that samples with FAM, CAL Fluor® Orange 560, CAL Fluor® Red 590, CAL Fluor® Red 610 and CAL Fluor®

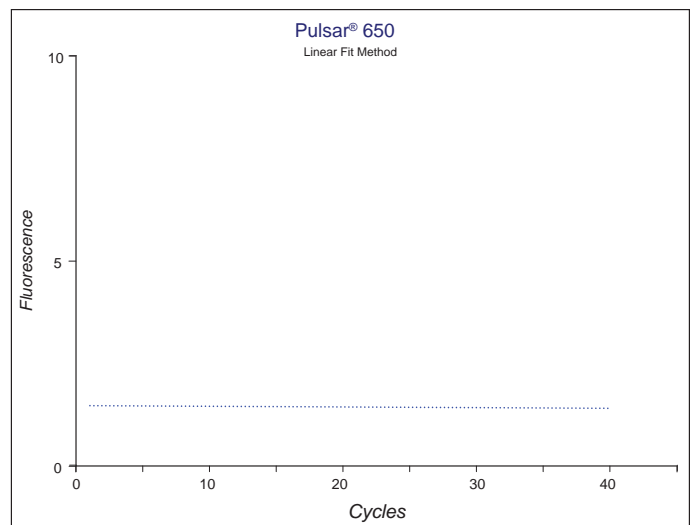
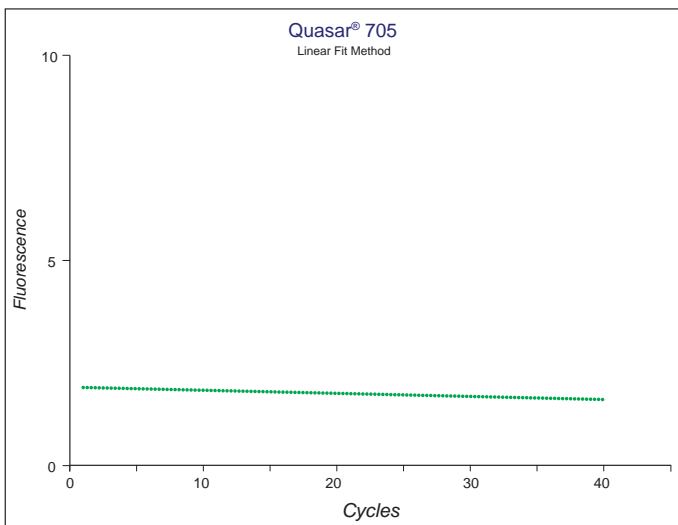
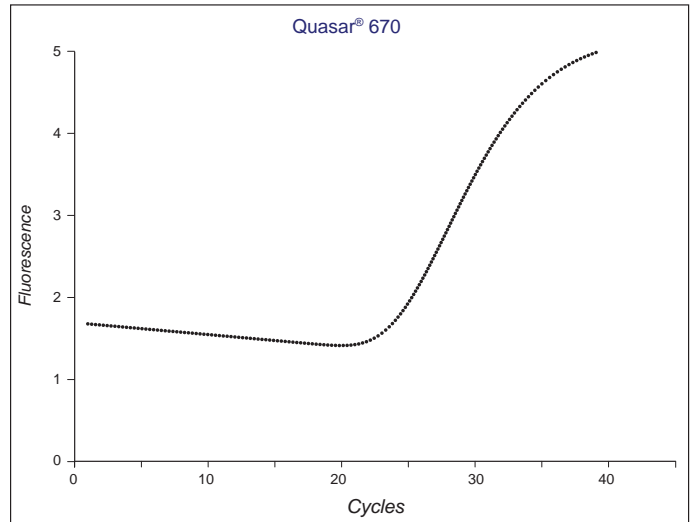
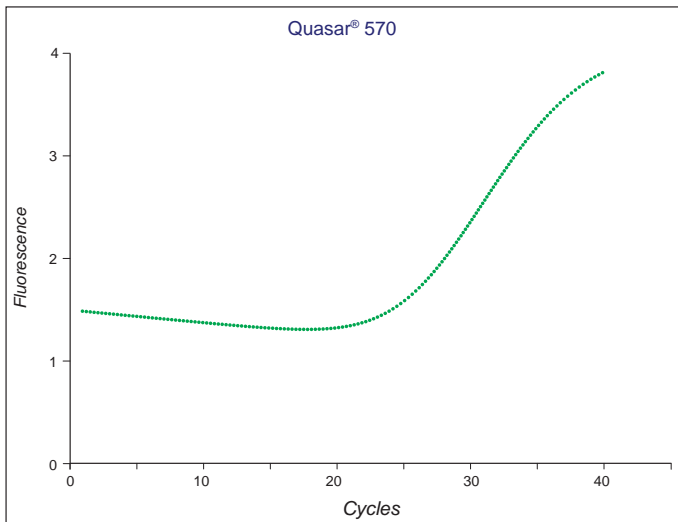
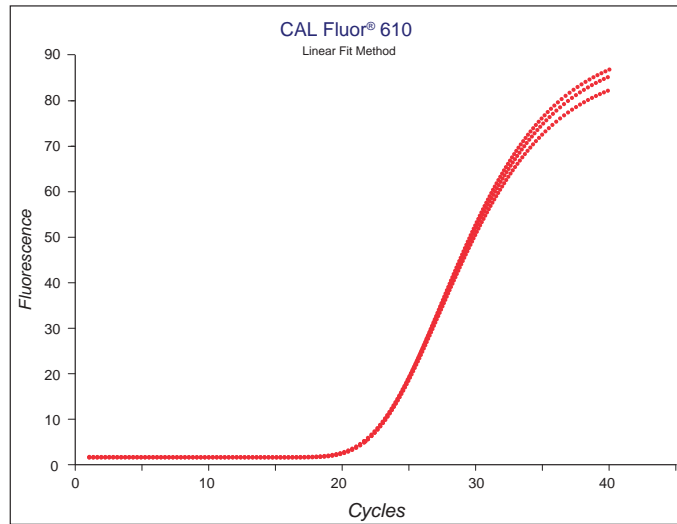
Red 635 produced detectable results, with threshold cycles (Ct) ranging from 21 to 24. Real-Time PCR reactions were not consistently detectable with Quasar® 570, 670 and 705 and Pulsar® 650, although PCR products were detected by electrophoresis (Figure 1). CAL Fluor® Orange 560 and CAL Fluor® Red 635 were further tested in order to assess channel cross-reactivity (Figure 2). By varying concentrations of probe, CAL Fluor® Red 590 was shown to have a detectable Ct with concentrations as low as 0.15 µM, but produces cross-talk between red and green channels. CAL Fluor® Red 635 was detectable with as little as 0.05 µM of probe.

A fluorophore was considered compatible for duplex reactions on the Spartan DX-12® with FAM if the average fluorescence ratio of the red channel/green channel was greater than 15 (Table 4).

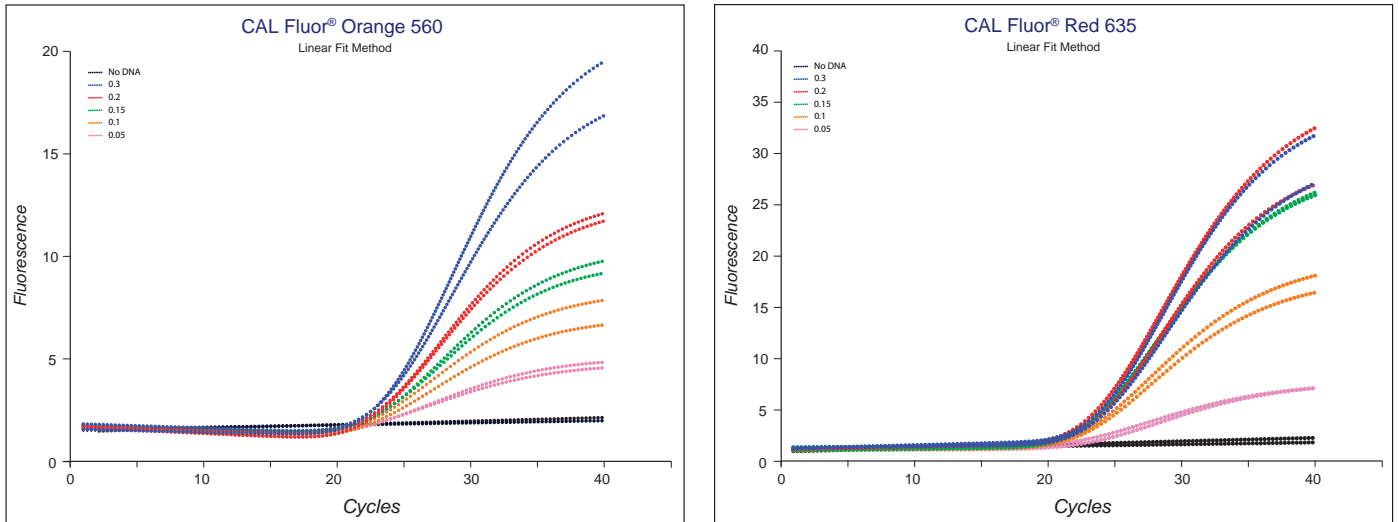
### Discussion and Conclusions

These results demonstrate that the Spartan DX-12





**Figure 1.** Real-Time PCR results for with nine different fluorophores.



**Figure 2.** Real-Time PCR results with probe dilutions for CAL Fluor® Orange 560 and CAL Fluor® Red 635.

Fluorophore	Singleplex		Duplex	
	Green	Red	Green	Red
FAM	Yes		Yes	
CAL Fluor® Orange 560		Yes		No
CAL Fluor® Red 590		Yes		No
CAL Fluor® Red 610		Yes		Yes
CAL Fluor® Red 635		Yes		Yes
Quasar® 570		No		No
Quasar® 670		No		No
Quasar® 705		No		No
Pulsar® 650		No		No

**Table 5.** Recommended fluorophores.

CAL Fluor® Orange 560 is capable of performing Real-Time PCR using TaqMan® probes with FAM, CAL Fluor® Orange 560, CAL Fluor® Red 590, CAL Fluor® Red 610 and CAL Fluor® Red 635 fluorophores. Other fluorophores predicted to be compatible with the Spartan DX-12® include: ROX™, HEX™, TAMRA™, LC Red 640 and Texas Red. Quasar® 570, 650, 670 and 705 not recommended for use with the Spartan DX-12®.

Table 5 lists fluorophores recommended for singleplex or duplex reactions with the Spartan DX-12®.

#### Disclaimer

PCR and real-time PCR processes are covered by patents issued and applicable in certain countries. This product is not licensed under these patents. Spartan does not encourage or support the unauthorized or unlicensed use of PCR or real-time PCR processes. Use of this instrument is recommended for persons that either have licenses to perform PCR and real-time PCR, or are not required to obtain licenses. Users interested in obtaining a license for these patents should contact the respective patent and license owners.

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