

Using Alternatives to VIC on the RainDrop® Digital PCR System

The RainDrop Digital PCR System allows users to pair a second probe in conjunction with FAM. While VIC is the most commonly paired second probe, this application note highlights 6 VIC alternatives and their intensity relative to VIC on the RainDrop Digital PCR System.

One of the strengths of the RainDrop Digital PCR System is the flexibility in the reagents that can be put into the drops. Whereas the RainDrop Digital PCR System has been shown to work very well with TaqMan® probes (Life Technologies), we realize that many people may want to utilize alternative probes, dyes and other unique chemistries. To address one of these aspects, we tested the System using different fluorescent dyes that can be used as alternatives to VIC). These dyes are available from a variety of vendors, including Integrated DNA Technologies (IDT), Eurogentec, AnaSpec and BioSearch Technologies amongst others. The probes we evaluated here are outlined in Table 1.

A summary of the reaction components is shown in Table 2. The concentration of Target 1 DNA and Target 2 DNA was kept consistent across each sample. A single FAM-based probe was used in each sample to amplify Target 1 DNA sequence. PMT2 probes were designed using the same

Target 2 DNA sequence, but with different fluorescent dyes. As can be seen in Figure 1, all of the dyes tested gave discernible clusters when duplexed with FAM. Of the dyes tested, Cal Fluor Orange (CFO-560), HEX and JOE produced droplets with less fluorescence than VIC. Three of the dyes, Yakima yellow, MAX and TET, produced droplets with fluorescence that was greater than VIC. An estimate of the absorption and emission peak for each dye is shown in Table 3.

Many factors influence the behavior of a fluorophore. These include (but are not limited to) composition of the master mix, age of the dye, sequence of the probe and the probe quencher. While differences between assays are expected, these results indicate that there are several different alternatives to VIC that work well on the RainDrop Digital PCR System.

Figure 1
Fluorescence Dye Test Results

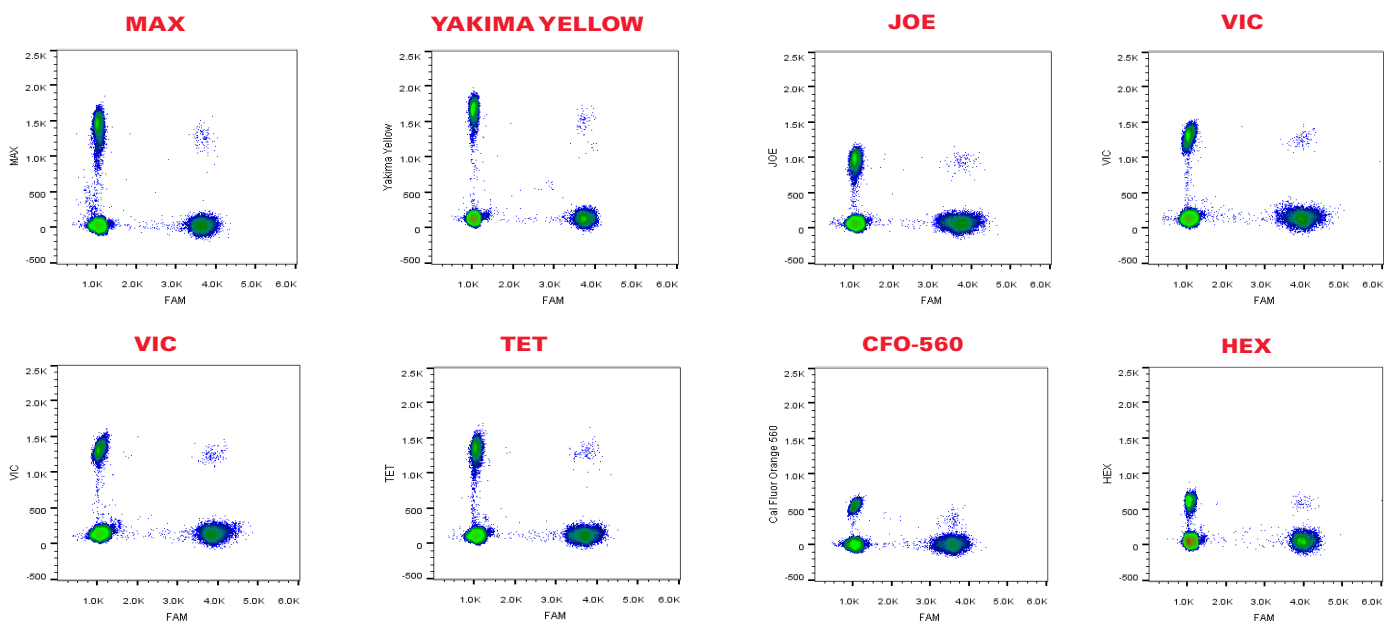


Table 1
Eclipse Double Dye

Fluor Dye (PMT2)	Probe Type	Manufacturer
VIC	TaqMan/TAMRA	Life Technologies
Yakima Yellow	Double-dye	Eurogentec/AnaSpec
MAX	PrimeTime Zen	Integrated DNA Technologies
TET	PrimeTime Zen	Integrated DNA Technologies
JOE	PrimeTime Zen	Integrated DNA Technologies
HEX	PrimeTime Zen	Integrated DNA Technologies
CAL Fluor Orange 560	BHQplus	Biosearch

Table 1

Table 2
Reaction Components Summary

Component	Final Concentration
TaqMan® Genotyping Master Mix	1 X
Target DNA 1	16,000 copies/rxn
Target DNA 2	8,000 copies/rxn
Target 1 Primers	900 nM
Target 2 Primers	900 nM
Probe 1 (FAM)	200 nM
Probe 2 (PMT2)	200 nM

Table 2

Table 3
Excitation and Emission Estimates

Dye	Abs max	Em max
CFO-560	538	559
HEX	535	556
JOE	522	550
VIC	538	554
TET	521	536
MAX	524	557
Yakima	530	549

Table 3

The RainDrop Digital PCR System is for Research Use Only; not for use in diagnostic procedures.

© 2013 RainDance Technologies, Inc. All rights reserved.

LCN 50-07053 Rev B