

Q qPCR Cycler Dye Detection

Provided in this Bulletin is a list of commonly used dyes for qPCR and the channels we can read them on. Most of them will be optimal for detection on a Q 4 channel instrument. The 2 channel option is primarily designed for users that typically work with SYBR Green all the time and do not require multiplexing.

The excitation is the energy pumped into the dye by an energy source. For the Q qPCR Cycler this is a high-powered LED. The emission is the fluorescence energy that is returned from the excited dye, which is picked up by a detector. For the Q qPCR Cycler this is a photodiode. Each channel will have its own excitation and emission (LED and photodiode). These sit side by side in a fixed optical path (see Figure 1) and work together to provide fast acquisition times (1 s). They are also very robust and so don't require calibration or any form of colour compensation or reference dyes. So even though the excitation and emission spectra are different numbers between the optical channel and the dye, the dye values are peaks and not a single value. The dyes have a very broad range of spectra. For the optical channels they have a band width that can be up to 20 nm wide. This ensures we can pick up a lot of different dyes but avoid cross talk, which is one dye's emission being captured by another non-specific channel. This is key when doing a multiplex qPCR as you don't want to be seeing false positives in your sample.

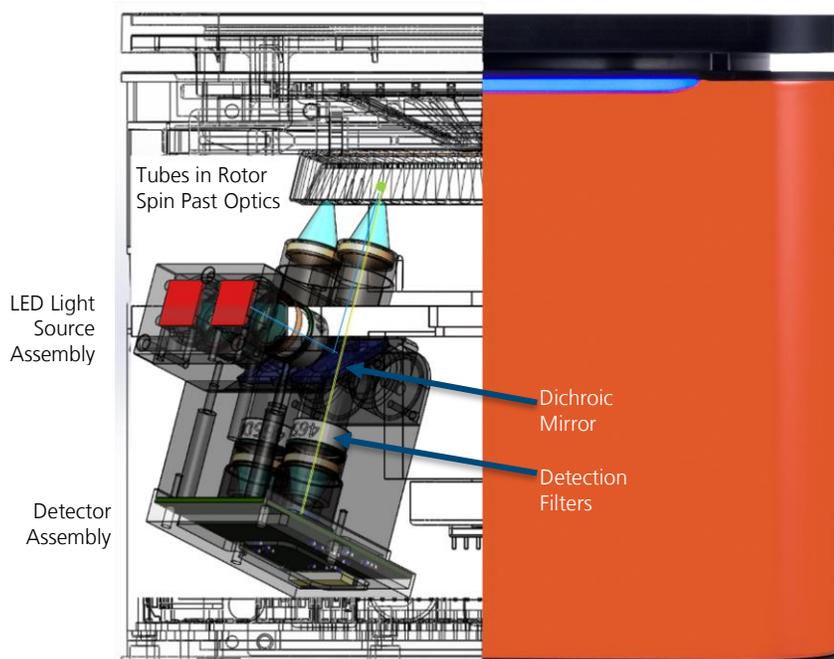


Figure 1

LED emits light, which passes through an excitation filter at a specific bandwidth. Dichroic mirror reflects the excitation light onto the tubes and then only allows the emission light to pass through to the detector via a specific emission filter.

Dye	Excitation	Emission	Channel	Application
BEBO	468	492		Intercalating
LC Green®	455	495		HRM dye
SYTO®	483	503		HRM dye
FAM™ (best)	494	515		Conjugated label
SYBR® Green I	494	521		Intercalating
RiboGreen®	500	520		RNA label
PicoGreen®	502	523		ds DNA label
Eva Green®	503	527		HRM dye
TET™	521	536	suboptimal	Conjugated label
CAL Fluor® Gold 540	522	541	suboptimal	Conjugated label
JOE™	520	548	suboptimal	Conjugated label
VIC®	538	554		Conjugated label
HEX™	535	555		Conjugated label
CAL Fluor Orange 560 (best)	540	561		Conjugated label
Quasar® 570	548	566		Conjugated label
Cy™3	550	570		Conjugated label
NED™	546	575		Conjugated label
TAMRA™	555	576		Conjugated label
CAL Fluor® Red 590	565	588	x	Conjugated label
ROX™	573	602		Conjugated label
Texas Red®	583	603		Conjugated label
CAL Fluor® Red 610 (best)	590	610		Conjugated label
LC® Red 640	620	635	suboptimal	Conjugated label
Quasar® 670 (best)	647	667		Conjugated label
Cy™ 5	651	674		Conjugated label
Cy™5.5	675	694		Conjugated label
Quasar® 705	690	705	x	Conjugated label