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USB® VeriQuest® SYBR® Green qPCR Master Mix (2X) with Fluorescein

Product number 75665

USB VeriQuest SYBR Green qPCR Master Mix with Fluorescein is a ready-to-use master mix optimized for SYBR Green detection on real-time PCR instruments that use fluorescein to perform dynamic well factor collection for data normalization (*e.g.* the iCycler iQ™, iQ5, or MyiQ™ real-time PCR systems).

The 2X master mix contains chemically-modified VeriQuest Taq DNA Polymerase, $MgCl_2$, ultrapure nucleotides with an optimized dUTP:dTTP ratio, Uracil-DNA Glycosylase (UDG), SYBR Green, and fluorescein in a proprietary reaction buffer.

The hot start Taq DNA polymerase has no polymerase activity prior to the initial heat activation step which allows reaction assembly at room temperature as well as higher specificity and sensitivity. Since the mix contains dUTP and UDG, carryover contamination prevention can be performed prior to amplification.

Storage conditions

-20°C for long-term storage. 4-8°C for short-term storage (≤ 3 months).

Brief protocol

1. Thaw the master mix and other frozen reagents at room temperature. Mix thoroughly, briefly spin to collect tube contents and then place on ice.

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2. Calculate the number of reactions to perform and assemble master mix and reaction plate on ice or at room temperature.
3. For each sample, add the following component volumes shown in the table below.

Components	**50 µl reaction volume**	**20 µl reaction volume**	**Final concentration**
VeriQuest SYBR Green qPCR Master Mix with Fluorescein (2X)	25 µl	10 µl	1X
10 µM Forward Primer	2.5 µl	1.0 µl	500 nM (range 150-900 nM)
10 µM Reverse Primer	2.5 µl	1.0 µl	500 nM (range 150-900 nM)
Template DNA	X µl	X µl	see below*
Water, PCR Qualified	up to 50 µl	up to 20 µl	----

*Optimal template input quantities: cDNA corresponding to 1 pg to 500 ng of total RNA. If template is cDNA from a first-strand synthesis reaction that has not been purified or diluted, do not exceed 10% of the final reaction volume (*i.e.* 2 µl for a 20 µl reaction). For genomic DNA, do not exceed 100 ng.

4. Seal plate with optically-transparent film, mix plate by gentle vortexing and then spin to collect contents without bubbles (*e.g.* 60 seconds at >2000 rpm).

5. Load the plate into the real-time PCR instrument and use the following recommended cycling conditions:

Standard cycling program for SYBR Green

	UDG treatment	Taq DNA polymerase activation and UDG inactivation	PCR amplification	
	Hold	Hold	35-45 cycles	
			Denature	Anneal/ Extend
Temperature	50°C	95°C	95°C	60°C
Time	2 minutes	10 minutes	15 seconds	30-60 seconds
Notes	*Optional for carryover contamination prevention*			Acquire real-time fluorescence data during this step

6. Addition of a Melt-Curve program and analysis is recommended to distinguish specific products from non-specific ones. Consult thermal cycler manual for details.
7. Analyze the results according to the recommended method for each instrument.