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# USB® VeriQuest™ Fast Probe qPCR Master Mix (2X)

## Product number 75680

VeriQuest Fast Probe qPCR Master Mix is a ready-to-use master mix optimized for TaqMan® probe detection on instruments using Fast mode cycling protocols that utilize ROX as a passive reference dye. The 2X mix contains hot start VeriQuest Fast Taq DNA Polymerase, $MgCl_2$, ultrapure nucleotides with an optimized dUTP:dTTP ratio, Uracil-DNA Glycosylase (UDG), and ROX Passive Reference Dye in a proprietary reaction buffer. The hot start Taq enzyme 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.
2. Calculate the number of reactions to perform and assemble master mix and reaction plate on ice or at room temperature.

*(continued on next page)*

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3. For each sample, add the following component volumes shown in the table below.

| Components | 20 µl reaction volume | Final concentration |
|---|---|---|
| VeriQuest Fast Probe qPCR Master Mix (2X) | 10 µl | 1X |
| 10 µM Forward Primer | 1.0 µl | 500 nM (range 150-900 nM) |
| 10 µM Reverse Primer | 1.0 µl | 500 nM (range 150-900 nM) |
| 10 µM TaqMan probe(s) | 0.5 µl | 250 nM (range 100-500 nM) |
| Template DNA | X µl | see below* |
| Water, PCR Qualified | 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:

**Fast cycling program for TaqMan Probes**

- Select Fast as the run mode
- Enter reaction volume
- Add the detectors as appropriate for probe(s) of interest
- Select ROX as the passive reference dye

| | 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 | 5 minutes | 3 seconds | 30 seconds |
| Notes | *Optional for carryover contamination prevention* | | | Acquire real-time fluorescence data during this step |

6. Analyze the results according to the recommended method for each instrument.