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VeriQuest™ SYBR™ Green One-Step qRT-PCR Master Mix (2X)

Product number 75705

VeriQuest SYBR Green One-Step qRT-PCR Master Mix is a ready-to-use master mix for real-time, quantitative analysis of RNA templates in a single reaction format. The master mix contains 2 tubes: VeriQuest 2X SYBR Green One-Step qRT-PCR Master Mix and VeriQuest 100X RT Enzyme Mix for SYBR Green Assay. The 100X RT Enzyme Mix is a blend of reverse transcriptase and RNase Inhibitor. The VeriQuest 2X SYBR Green One-Step qRT-PCR Master Mix contains chemically-modified VeriQuest Taq DNA Polymerase, ultrapure nucleotides, SYBR Green I and ROX™ Passive Reference Dye in an optimized buffer formulation for quantitative, real-time reverse transcription PCR detection with SYBR Green (qRT-PCR).

Brief protocol

This protocol applies to a single reaction where RNA template, primers, and water are added to the VeriQuest SYBR Green One-Step qRT-PCR Master Mix. For multiple reactions, increase the volumes of the reaction components proportionally.

1. Thaw the master mix and other necessary frozen reagents at room temperature. Keep RT Enzyme Mix and RNA sample on ice. Mix thoroughly, briefly spin to collect tube contents and then place on ice. The RNA samples should always be kept on ice.
2. Assemble reaction tubes or plates on ice.
3. The table below shows recommended component volumes. Prepare the reaction volume that is appropriate for your real-time PCR instrument.

   It is highly recommended to make a master mix for at least 10 reactions to reduce pipetting errors.

Components	50 µl reaction volume	20 µl reaction volume	Final concentration
VeriQuest SYBR Green One-Step qRT-PCR Master Mix (2X)	25 µl	10 µl	1X
VeriQuest 100X RT Enzyme Mix for SYBR Green Assay	0.5 µl	0.2 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 RNA	X µl	X µl	as needed, <1 µg†
RNase Free Water, DEPC Treated	up to 50 µl	up to 20 µl	NA

*Optimal primer concentration is 0.2 µM. In order to avoid primerdimers and non-specific products, use ≤ 0.5 µM. Because the reverse primer is also used during the initial reverse transcription step, it may be helpful to double the amount of reverse primer only.
†Total RNA may be used at 1 pg to 100 ng and poly(A)+ mRNA may be used at 100 fg to 100 ng per reaction.

4. Cap tubes or seal plates with optically clear caps or film. Mix tubes or plates by gentle vortexing and then spin to collect contents without bubbles (*e.g.* 2-5 minutes at 1000-2000 x *g*).

5. The following table shows recommended cycling conditions:

Standard cycling program

1 cycle of: 50°C for 10 minutes: Reverse transcription of RNA by reverse transcriptase to generate cDNA.
1 cycle of: 95°C for 10 minutes: VeriQuest Taq DNA Polymerase activation and reverse transcriptase inactivation
35-45 cycles of: 95°C for 15 seconds 60°C for 30 seconds: Acquire real-time fluorescence data during this step.
Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult the thermalcycler manual for details.

6. If desired, confirm that specific RT-PCR products have been generated by agarose gel electrophoresis. Amplicons may be detected on gels with ethidium bromide or using the SYBR Green I from the reaction mix.

Optional protocol

Running a no-RT control

This protocol applies when a no-RT control is desired. A no-RT control reaction allows you to identify potential genomic DNA detection during your one-step real-time PCR reaction. To run a no-RT control: Prepare a reaction using the table above without the addition of VeriQuest 100X RT Enzyme Mix for SYBR Green Assay. This will eliminate the reverse transcriptase from the master mix while still retaining the necessary PCR amplification components.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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