qScript XLT One-Step RT-qPCR ToughMix, low ROX is a ready-to-use, highly sensitive master mix for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5’-hydrolysis probes on Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, or StepOnePlus™ instruments. First-strand cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. It is ideal for highly sensitive quantification of RNA viruses or low abundance RNA targets as well as high throughput gene-expression studies. The system has been optimized to deliver maximum RT PCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times. qScript XLT One-Step RT-qPCR ToughMix, low ROX contains all required components for RT-qPCR except RNA template and probe. It is compatible with all dual-labeled probe chemistries.

qScript XLT is an engineered M-MLV reverse transcriptase with reduced RNase H activity and improved activity and stability at higher temperatures. The use of higher temperatures (50 to 55°C) for the first-strand step of one-step RT-qPCR provides higher specificity for primer annealing and disruption of RNA secondary structure that can interfere with cDNA synthesis.

**Product Specifications**

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<th>Assay</th>
<th>1-Step RT qPCR Functional Assay</th>
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**Component Part Numbers:**

84227: qScript XLT One-Step RT-qPCR ToughMix, Low ROX (2X)

**Quality Control and Analyses:**

**1-Step RT-qPCR Functional Assay:** Real-time One-Step RT-PCR of a reference gene (ACTB) in triplicate reactions is performed on a 10 fold serial dilution over 6 orders of dynamic range (1 μg to 1 pg) using a Universal Reference total RNA preparation. Cq standard curve analysis must have coefficient of determination ($r^2$) ≥0.995 with a slope between −3.20 and −3.65. Control reactions lacking template RNA (NTC) must remain below fluorescence threshold through 45 PCR cycles.

**Nuclease Assay:**

DNase: DNase activity must be below the detectable limits of 100 pg DNase I equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component at 1X concentration.

RNase: RNase activity must be below the detectable limits of 1 pg RNase A equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component at 1X concentration.

**Notes:**

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This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

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