

qScript™ XLT One-Step RT-PCR Kit

Tough RT-PCR for Tough Samples

The qScript XLT One-Step RT-PCR kit is for highly sensitive, robust one-step RT-PCR even in the presence of PCR inhibitors often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues.



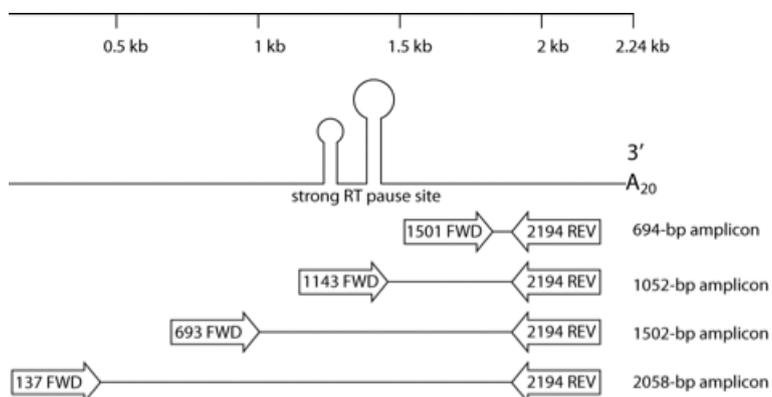
Quanta
BIOSCIENCES™

FEATURES AND BENEFITS

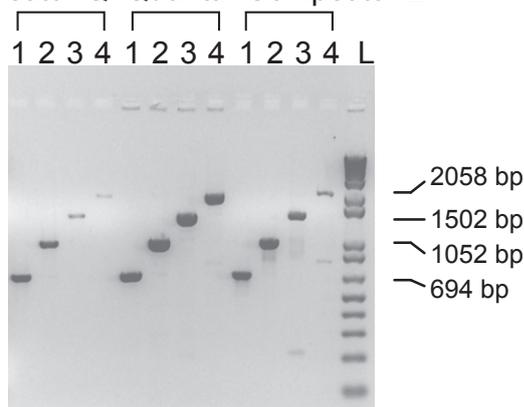
- Tough tested to overcome inhibitors
- Optional Gel Track Loading Dye included
- Superior reproducibility
- Convenient single tube reaction assembly at room temperature

The qScript XLT One-Step RT-PCR Kit is a convenient and highly sensitive system for amplification of RNA templates up to 2 kb. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. This system has been optimized to deliver maximum RT PCR efficiency, sensitivity, and specificity.

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 (48 to 55°C) for the cDNA synthesis step of one-step RT-PCR provides higher specificity for primer annealing and disruption of RNA secondary structure that can interfere with cDNA synthesis. The enzyme is supplied as a mixture with ribonuclease inhibitor protein to protect the integrity of RNA templates in crude lysates or samples where RNase contamination may limit assay sensitivity.



Competitor Q Quanta Competitor L

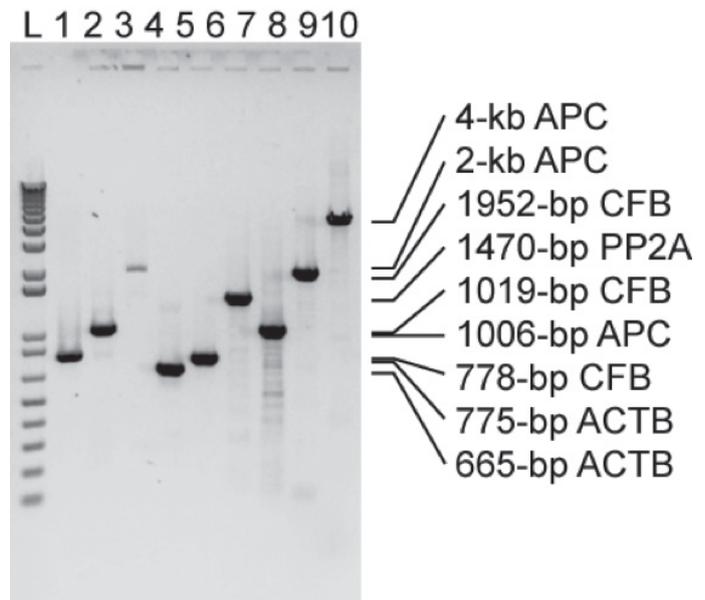


1-Step RT-PCR of varying length amplicons from 2.2-kb TcR in vitro transcript RNA

Each kit was used according to the manufacturer's recommended procedure in 20- μ l reaction volumes containing 200 μ M each primer and 1 x 10⁵ copies of an in vitro synthesized run-off transcript for the tetracyclin resistance gene (TcR), produced using T7 RNA polymerase. Following first-strand synthesis and activation of the hot-start Taq polymerase, all reactions were amplified for 30 cycles of 94°C, 15s; 60°C, 20s; 72°C, 2 min followed by a final hold of 5 min at 72°C. 1/5th of each reaction was analyzed on a 0.8% agarose, 0.5X TBE gel containing 0.25 mg/mL ethidium bromide.

A key component of the system is the One-Step ToughMix®. This master mix is highly resistant to PCR inhibitors and contains an ultra pure, hot-start, highly processive thermostable DNA polymerase that is blended with a proof-reading (3'-exonuclease) polymerase for improved PCR fidelity and fragment lengths. High-avidity monoclonal antibodies provide an extremely stringent automatic hot-start that minimizes the potential for primer-dimer and other non-specific PCR artifacts without compromising polymerase activities. Highly specific amplification is crucial to successful RT PCR as non-specific product(s) can compete with amplification of the target sequence and impair PCR efficiency. The proprietary reaction buffer has been specifically formulated to maximize activities of both the reverse transcriptase and thermostable DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts.

GelTrack® Loading Dye is a mixture of blue and yellow electrophoresis-tracking dyes that migrate at approximately 4kb and 50 bp. This optional component simplifies post PCR analysis, allowing direct loading of RT-PCR product on agarose gels following amplification. The GelTrack Loading Dye solution is not included with the sample kit.



1-Step RT-PCR of varying length fragments from HeLa cell total RNA

RT-PCR program: 48C 20min; 94C 3min; 94C 15sec; 60C 15 sec; 68C 2min; 35 cycles

ACTB = 2 ng HeLa total RNA, all others 20 ng HeLa total RNA

Load 5 uL of 20 uL rxn on 0.8% gel

(CFB = Complement Factor B, PP2A = protein phosphatase 2A, ACTB = beta actin, APC = adenomatous polyposis coli)

COMPONENTS

Reagent	Description	Quantity
qScript XLT One-Step Reverse Transcriptase (25X)	25X concentrated mixture of qScript XLT reverse transcriptase and recombinant ribonuclease inhibitor protein.	1 x 200 µL
One-Step ToughMix (2X)	2X concentrated reaction buffer containing dNTPs, magnesium chloride, hot-start DNA polymerase, and stabilizers	2 x 1.25 mL
GelTrack Loading Dye (50X)	50X concentrated mixture of RT-PCR compatible, blue and yellow electrophoresis-tracking dyes	1 x 0.4 mL
Nuclease-free water		2 x 1.5 mL

ORDERING INFORMATION

PRODUCT	Quanta Cat. No.	Pack Size (20uL Reactions)
qScript XLT One-Step RT-PCR Kit	95143-200	200 x 25 µL reactions