



qScript® XLT One-Step RT-PCR Kit

Cat. No 95143-020 Size: 20 x 25- μ L reactions
95143-200 200 x 25- μ L reactions

Store at **-25°C to -15°C**

Description

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.

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 size 95143-020

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 μ L
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

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.

Repeated freezing and thawing of the ToughMix will not affect product performance.

For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for One-Step RT-PCR

- Thaw components at room temperature. Mix thoroughly by gently vortexing, and then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.
- To maximize specificity, reactions should be assembled on ice. The thermostable DNA polymerase is inactive prior to high temperature activation; however, qScript XLT reverse transcriptase is active at lower temperatures. During reaction assembly, equilibrate the thermocycler block to 50°C (or your preferred temperature for cDNA synthesis) and pause the run. Transfer fully assembled reactions from ice to the pre-warmed thermocycler and resume the run.
- First-strand synthesis can be carried out between 42°C and 55°C. cDNA conversion of short fragments (under 500 nt) is complete within 5 to 10 minutes. As a general starting point, use a 20-minute incubation at 48°C. We recommend a 3 minute incubation at 94°C to fully inactivate the RT prior to PCR cycling.
- Perform a minus RT control to verify that amplification product is derived from RNA sequence and not genomic DNA.

Guidelines for One-Step RT-PCR Continued:

- Primer concentrations may need to be optimized for different target sequences. qScript XLT reverse transcriptase generally prefers 200 to 600 nM primer for effective priming of the cDNA synthesis step. We recommend using 400 nM each primer as a general starting point. The optimal concentration of each primer may need to be empirically determined. We suggest testing a range between 100 and 800 nM. Lower primer concentrations (150 to 250 nM) often reduce non-specific products, should they occur. Higher primer concentrations (400 to 500 nM) can be used to drive higher product yields for longer fragments.
- Suggested input quantities of template are: 1 pg to 1 µg total RNA; 10 fg to 100 ng poly A(+) RNA; 10 to 1x10⁸ copies virus RNA.
- When performing multiple reactions, always prepare a reaction cocktail that contains all components except RNA template. After mixing, dispense the reaction cocktail (15 to 20-µL volume / reaction) to each tube and then add the RNA template (5 to 10-µL volume / reaction) as a final component. Allow for extra reactions (5-10%) to accommodate pipetting steps when assembling the reaction cocktail.
- Thoroughly mix fully assembled reactions by gently vortexing, and then briefly centrifuge to collect contents.

Reaction Assembly (on ice)

Component	Volume for 25-µL rxn.	Reaction Cocktail for 96 rxns (100)	Final Concentration
One-Step ToughMix (2X)	12.5 µL	1250 µL	1X
Forward primer (100 µM)	Variable (0.05 to 0.15 µL)	10 µL (for 400 nM)	200 – 600 nM
Reverse primer (100 µM)	Variable (0.05 to 0.15 µL)	10 µL (for 400 nM)	200 – 600 nM
GelTrack Loading Dyes (50X)	0.5 µL	50 µL	1X (optional)
qScript XLT One-Step RT (25X)	1 µL	100 µL	1X
Nuclease-free water	Variable	80 µL	
<u>RNA template</u>	<u>5 – 10 µL</u>		variable
Final Volume (µL)	25 µL	1,500 µL	

Dispense 15 µL to each rxn.
Then add 10 µL RNA template

Note: For larger or smaller reaction volumes scale all components proportionally. Omit qScript XLT One-Step RT for minus RT control reactions.

Reaction Protocol

Incubate reactions as follows:

cDNA Synthesis	48°C, 20 min
Initial denaturation	94°C, 3 min
PCR cycling (30 - 45 cycles)	
Denaturation:	94°C, 10 to 20s
Annealing:	55 – 65°C, 20 to 60s
Extension:	68°C to 72°C, 1 minute per kb of target length

Quality Control

Kit components are free of contaminating DNase and RNase. The qScript XLT One-Step RT-PCR Kit is functionally tested in RT-PCR for amplification of a 2-kb fragment of the APC gene from HeLa cell total RNA.

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