qScript® Ultra Flex Kit

Cat No.	95215-025 95215-100	Size:	25 x 20 µL reactions (1x 0.1 mL) 100 x 20 µL reactions (1x 0.4 mL)	**Store at -25°C to -15°C**

Description

The qScript Ultra Flex Kit is a complete, next-generation system that delivers rapid and efficient first-strand cDNA synthesis while providing flexibility in the choice of priming method. The kit features a stabilized 5x-concentrated reaction mix that provides all components for first-strand synthesis except RNA template and primers. A key component is a novel, state-of-the-art, RNase H deficient reverse transcriptase (RT) that was engineered for improved thermostability, velocity, processivity, and resistance to many common reaction inhibitors.

The superior performance of this novel RT is further supported by proprietary replication accessory proteins and a recombinant mammalian RNase inhibitor protein. These features allow for reactions to be carried out at higher temperatures than standard reverse transcriptases, improving sensitivity and minimizing potential interference from blocking secondary structures. In addition, the improved synthesis speed and enhanced processivity allow for reactions to be complete in 10 minutes, with high yields of full-length cDNA products as long as 20 kb.

The kit is supplied with both an anchored oligo(dT) solution and a modified random primer mixture that enhances cDNA yield with low input quantities or compromised RNA samples. Primer solutions include a proprietary enhancer compound that improves cDNA priming efficiency and protects RNA integrity during the optional denaturation step to destabilize RNA secondary structures. This enhancer is provided as a separate solution for use with your gene-specific primers. The kit is compatible with total RNA, polyA+ RNA, or viral RNA. The resulting cDNA product is directly compatible with real-time RT-qPCR methods or end-point RT-PCR. The length of cDNA product is dependent on the priming strategy and the quality of the RNA template. Greater length cDNA products can be obtained from oligo(dT) priming, in which primers specifically anneal to the 3′ poly(A) tails of mRNA, or by using gene-specific priming, in which primers anneal to a defined sequence. While strategies that utilize random primers produce shorter first strand cDNA, they are suitable for obtaining larger yields of product from the 5′-ends of RNA molecules and from classes of RNA biotypes that do not contain a 3′ poly(A) tail. Complete, unbiased first strand cDNA synthesis can be obtained with a mixed primer strategy, in which oligo(dT) and random primers are combined.

Components

	95215-025	95215-100
5x qScript Ultra Reaction Mix Optimized master mix containing buffer, magnesium, dNTPs and qScript Ultra RT	1 x 100 µL	1 x 400 µL
Oligo(dT) primers 10x concentrated in Enhancer solution	1 x 50 µL	1 x 200 µL
Random primers 10x concentrated in Enhancer solution	1 x 50 µL	1 x 200 µL
GSP Enhancer (10x)	1 x 50 µL	1 x 200 µL
Nuclease-free Water	1 x 1.5 mL	2 x 1.5 mL

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C.
After thawing, mix thoroughly before use.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Standard Reaction Protocol

1. Thaw components, mix and centrifuge before use. Hold on ice before use.
2. Add the following to a thin-walled PCR tube or reaction plate on ice:

Component	Volume for 20 µL rxn.	Final Concentration
Nuclease-free water	variable	
Template RNA	variable	2.5 µg to 1 pg total RNA
10x Oligo(dT) or 10X Random Primers or 1-10 µM Gene-specific primer(s)	2 µL	1x Oligo(dT) or 1x Random primers or 0.1-1 µM Gene-specific primers
(Optional) 10x GSP Enhancer, if using Gene-specific primer(s)	2 µL	
5x qScript Ultra Reaction Mix	4 µL	
Final volume	20 µL	

NOTE: For multiple reactions, a master mix can be prepared with all components except template RNA and dispensed into 96-well plates or PCR tubes.

3. Mix by gentle vortexing, then briefly centrifuge to collect contents.
4. Incubate for:
    - 5-10 minutes at 25°C (only required if using random primers)
    - 10 minutes at 55°C
    - 5 min at 85°C
    - Hold at 4°C
5. After the completion of cDNA synthesis, reactions can be used directly for endpoint RT-PCR or RT-qPCR analysis. It is recommended that PCR reactions contain no more than 1/5 volume of the first-strand cDNA reaction. If desired, reactions can be diluted with TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Reaction can be stored at -20°C for future use.

Two-Phase Reaction Protocol

For improved yield of some long cDNA products, or if template is known to contain regions of secondary structure

1. Thaw components, mix and centrifuge before use. Hold on ice before use.
2. Add the following to a thin-walled PCR tube or reaction plate on ice:

Component	Volume for 20-µL rxn.	Final Concentration
Nuclease-free water	variable	
Template RNA	variable	2.5 µg to 1 pg total RNA
10x Oligo(dT) or 10X Random Primers or 1-10 µM Gene-specific primer(s)	2 µL	1X Oligo(dT) or 1X Random primers or 0.1-1 µM Gene-specific primers
(Optional) 10x GSP Enhancer, if using Gene-specific primer(s)	2 µL	
Final volume	16 µL	

3. Mix by gentle vortexing, then briefly centrifuge to collect contents.
4. Incubate for 5 min at 65°C then immediately transfer to 4°C.
5. Add to each reaction mixture:

Component	Volume for 20-µL rxn.	Final Concentration
5x qScript Ultra Reaction Mix	4 µL	1X
Final Volume (µL)	20 µL	

6. Mix by gentle vortexing, then briefly centrifuge to collect contents.
7. Incubate for:
    - 5-10 minutes at 25°C (only required if using random primers)
    - 10 minutes at 55°C
    - 5 min at 85°C
    - Hold at 4°C
8. After the completion of cDNA synthesis, reactions can be used directly for endpoint RT-PCR or RT-qPCR analysis. It is recommended that PCR reactions contain no more than 1/5 volume of the first-strand cDNA reaction. If desired, reactions can be diluted with TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Reaction can be stored at -20°C for future use.

Quality Control

Functional PCR Assay for Flex Kit Reaction Mix (5X): Detection of ß-actin mRNA from 100 ng to 100 fg of total RNA. Coefficient of determination (R2) ⩾ 0.990 with a slope analysis between -3.20 and -3.70. Single bands visible at 9 kb and 15 kb from 30 cycles of PCR using 100 ng Oligo-dT cDNA.

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