Quanta BIOSCIENCES™

qScript™ Flex cDNA Synthesis Kit

Cat Nos.		Size		
	95049-025		25 x 20-µL reactions	**Store at -20°C**
	95049-100		100 x 20-µL reactions	

Description

The qScript Flex cDNA Synthesis Kit is an easy-to-use and highly efficient kit for the synthesis of first-strand cDNA that enables your choice of cDNA priming method. The kit provides optimized reagents for priming with oligo dT, random primer, gene-specific primer (GSP), or any combination thereof. A key component is a proprietary enhancer compound that improves cDNA priming efficiency. This is provided as a separate solution for use with your gene-specific primers, or pre-blended with the supplied Oligo dT or Random Primer solutions.

qScript reverse transcriptase is a mixture of an engineered RNase H^+ MMLV RT and recombinant ribonuclease inhibitor protein that has been specifically optimized for use with the provided qScript Flex Reaction Mix. This unique 5X master mix of buffer, magnesium, stabilizers and dNTPs simplifies reaction assembly and ensures robust and reproducible synthesis of first strand product from 10 pg to 1µg of total RNA or purified $polyA^+$ RNA template.

The resulting cDNA product is directly compatible with current real-time RT-PCR methods or end-point RT-PCR. Length of cDNA product is dependent upon priming strategy and quality of the RNA template. Oligo dT or GSP can be used for long RT-PCR of RNA targets up to 12 kb. Random primer is suitable for RT-PCR of RNA targets less than 1 kb. Additionally, the location of the target sequence in the mRNA should be considered when selecting a priming method. Oligo dT primer is the preferred choice for RT-PCR of 3'-end sequences. The efficacy of RT-PCR of 5'-end regions is often improved by using random or gene-specific primers.

Any of the three priming methods is suitable for real-time quantitative RT-PCR (qRT-PCR). However, we recommend using a mixture of the Oligo dT and Random Primer to achieve consistent and unbiased first-strand synthesis over a broad range of RNA template concentrations. This priming strategy has been incorporated into our qScript cDNA SuperMix (95048-025, 95048-100, 95048-500) and qScript cDNA Synthesis Kit (95047-025, 95047-100, 95047-500). These kits provide streamlined optimized solutions for gene-expression studies using real-time RT-PCR. For more information please visit our web site at www.quantabio.com.

Components

	95049-025	95049-100
qScript Flex Reaction Mix (5X) 5X concentrated solution of optimized buffer, magnesium, dNTPs, and stabilizers	1 x 100 µL	1 x 400 µL
Oligo dT 10X concentrated solution of $oligo(dT)_{20}$ with GSP Enhancer	1 x 50 µL	1 x 200 µL
Random Primer 10X concentrated solution of random primer with GSP Enhancer	1 x 50 µL	1 x 200 µL
GSP Enhancer (10X)	1 x 50 µL	1 x 200 µL
qScript Reverse Transcriptase	1 x 25 µL	1 x 100 µL
Nuclease-free water	1 x 1.5 mL	1 x 1.5 mL

Storage and Stability

Kit components are stable for one year when stored in a constant temperature freezer at -20°C. After thawing, mix thoroughly before using.

Reaction Protocol for Oligo dT or Random Primer

1. Thaw all components (except enzyme), mix thoroughly, and centrifuge before use. Hold on ice before use.
2. Add the following to a 0.2-mL thin-walled PCR tube or 96-well PCR reaction plate sitting on ice:

Component	volume
RNA (1 µg to 10 pg total RNA)	variable
Nuclease-free water	variable
Oligo dT or Random Primer	2 µL
final volume	15.0 µL

Note: Use 2 µL of Random Primer and 2 µL of Oligo dT for a mixed priming strategy. For multiple first-strand reactions, prepare a master mix with the reaction mix and RT and dispense 5 µL into each tube.

3. Mix components by gently vortexing and then centrifuge 10s to collect contents.
4. Incubate for 5 min at 65°C and then snap chill in ice.

Reaction Protocol for Oligo dT or Random Primer continued:

5. Add the following to the primed RNA template mixture:

Component	volume
qScript Flex Reaction Mix (5X)	4 µL
qScript Reverse Transcriptase	1 µL
final volume	20.0 µL

> Note: For multiple first-strand reactions, prepare a master mix with the reaction mix and RT and dispense 5 µL into each tube.

6. Mix components by gently vortexing and then centrifuge 10s to collect contents.
7. Incubate:

Random Primers	Oligo(dT)$_{20}$ Primer
10 min at 25ºC	-
45 min at 42ºC	60 to 90 min at 42ºC
5 min at 85ºC	5 min at 85ºC
Hold at 4ºC	Hold at 4ºC

8. After completion of cDNA synthesis, use 1/5th to 1/10th of the first-strand reaction (2-4 µL) for PCR amplification. Dilution of the first-strand reaction with TE buffer [10 mM Tris (pH 8.0), 0.1 mM EDTA] is useful for addition of larger volumes (5 – 10 µL) to the PCR, or when storing the material for future use. cDNA may be stored at -20ºC

Reaction Protocol for Gene-Specific Priming

1. Thaw all components (except enzyme), mix thoroughly, and centrifuge before use. Hold on ice before use.
2. Add the following to a 0.2-mL thin-walled PCR tube or 96-well PCR reaction plate sitting on ice:

Component	volume	
RNA (1 µg to 10 pg total RNA)	variable	
Nuclease-free water	variable	
gene-specific primer (10 to 20 pmol)	variable	(0.5 to 1 µM final conc., 20 µL vol.)
GSP Enhancer	2 µL	
final volume	15.0 µL	

3. Mix components by gently vortexing and then centrifuge 10s to collect contents.
4. Incubate for 5 min at 65ºC and then immediately transfer to 42ºC.
5. Add:

Component	volume
qScript Flex Reaction Mix (5X)	4 µL
qScript Reverse Transcriptase	1 µL
final volume	20.0 µL

> Note: For multiple first-strand reactions, prepare a master mix with the reaction mix and RT and dispense 5 µL into each tube.

6. Mix components by gently vortexing and then incubate:

   30 to 60 min at 42ºC
   5 min at 85ºC
   Hold at 4ºC

7. After completion of cDNA synthesis, use 1/5th to 1/10th of the first-strand reaction (2-4 µL) for PCR amplification. Dilution of the first-strand reaction with TE buffer [10 mM Tris (pH 8.0), 0.1 mM EDTA] is useful for addition of larger volumes (5 – 10 µL) to the PCR, or when storing the material for future use. cDNA may be stored at -20ºC

Quality Control

Real-time qRT-PCR of a reference gene is performed on first-strand product from both random-primed and oligo dT-primed cDNA. First-strand synthesis is performed in triplicate on each dilution of a log-fold serial dilution of HeLa cell total RNA from 1 pg to 1 µg. Standard curve analysis must generate a slope between -3.250 and -3.650 with a correlation coefficient between -0.995 and -1.000.

Long-RT-PCR of an 8-kb fragment from the APC gene must produce a discrete and visible product by agarose gel electrophoresis following a 30-cycle amplification using 1/10th of the first-strand product from 1 µg of HeLa cell total RNA and oligo dT primer.

All kit components are free of contaminating DNase or RNase.

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