

# qScript<sup>®</sup> Ultra Flex Kit

## Faster, longer, tougher next generation cDNA synthesis with flexible priming

### FEATURES AND BENEFITS:

- Enhanced Thermostability – Engineered for RT activity up to 65°C, overcoming challenging secondary structures
- Superior Speed & Ease of Use – 10 minute reaction time with fewer pipetting steps
- Maximum Yield & Sensitivity – Improved cDNA yield with limiting or compromised samples
- Ultimate Inhibitor Resistance – Overcome a wide array of PCR inhibitors (salt, heparin, hematin, etc.)
- Longer Transcripts – Ability to reverse transcribe single targets up to 20 kb from total RNA samples

### DESCRIPTION:

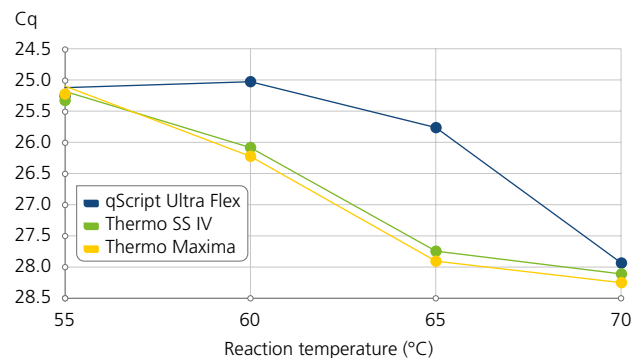
qScript Ultra Flex Kit is easy-to-use and highly efficient for the synthesis of first-strand cDNA with flexible priming methods including oligo-dT, random primers and gene specific priming to reverse transcribe RNA to cDNA. A key component is a novel, state-of-the-art, RNase H deficient reverse transcriptase engineered for improved thermostability, velocity, processivity, and resistance to many common reaction inhibitors. qScript Ultra Flex Kit contains all required components for first-strand cDNA synthesis except RNA template and gene specific primers. The cDNA product is compatible with downstream 2-step RT-qPCR or RT-PCR procedures.

### Enhanced Thermostability & Superior Speed

qScript Ultra Flex Kit has an optimal reaction temperature of 55°C and a 10 minute reaction time, significantly reducing time to result while working through challenging secondary RNA structures. The lower C<sub>q</sub> values for the engineered qScript Ultra reverse transcriptase indicate more efficient cDNA synthesis at high temperatures, critical for progressing through high GC-regions and regions of RNA secondary structure.

Product	1 <sup>st</sup> Temp	Time	2 <sup>nd</sup> Temp	Time	Total Time
qScript Ultra Flex	55°C	10 min	85°C	5 min	15 min
SuperScript <sup>™</sup> IV	55°C	10 min	80°C	10 min	20 min
Maxima <sup>™</sup>	50°C	15 min	85°C	5 min	20 min

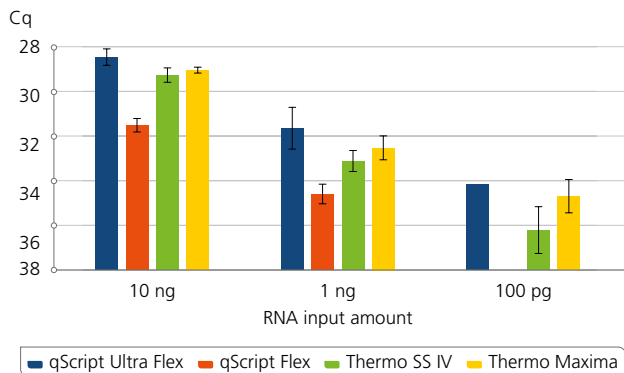
**Table 1 Reaction Time Comparison.** Incorporating the highly rapid and processive qScript Ultra reverse transcriptase, the qScript Ultra Flex Kit requires only 10 minutes for first-strand cDNA synthesis over a broad range of input RNA quantities, even for very long cDNA products. Total reaction time including heat inactivation is only 15 minutes.



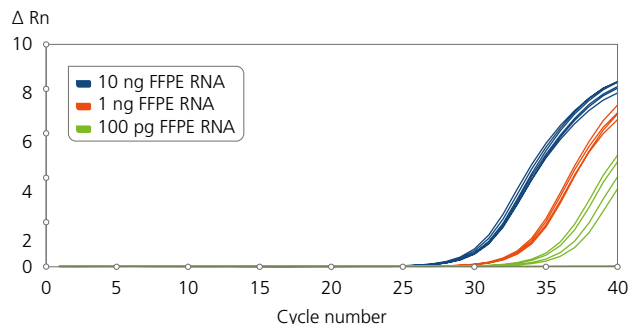
**Figure 1 Thermostability and Activity at High Temperatures.** cDNA was synthesized in reactions using 5 ng total human RNA and a stable LDHA gene-specific reverse primer. Each were incubated at the indicated temperatures, followed by reaction termination with products detected by qPCR. The lower C<sub>q</sub> values for the engineered qScript Ultra reverse transcriptase indicate more efficient cDNA synthesis at high temperatures up to 65°C, with no loss in performance up to 60°C, critical for progressing through high GC-regions and regions of RNA secondary structure.

## Maximum Yield and Sensitivity

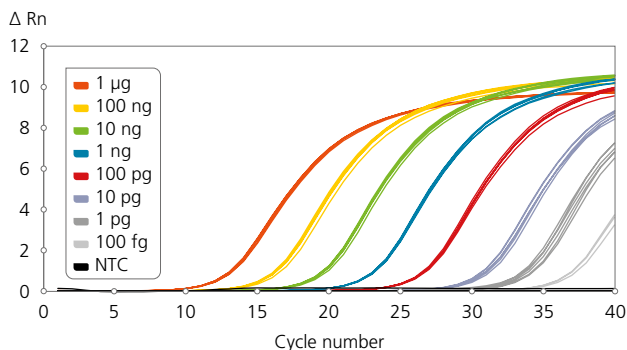
qScript Ultra Flex Kit provides higher yield across various RNA input amounts. The sensitivity of the kit enables low input RNA quantities or challenging samples to be detected while maintaining linearity in cDNA synthesis.



**Figure 2 Yield Comparison.** In random-primed first-strand cDNA synthesis reactions, the qScript Ultra Flex Kit improves RNA conversion and allows for detection of sequences from highly degraded RNA isolated from FFPE liver tissue.



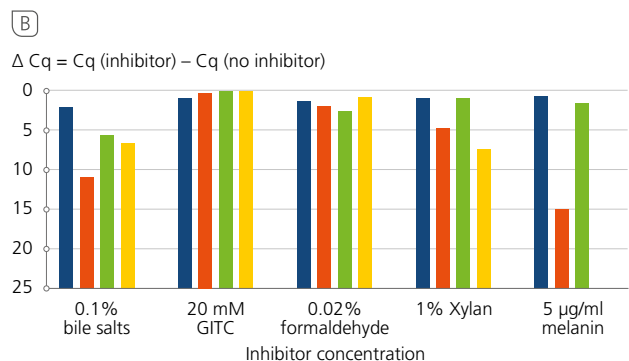
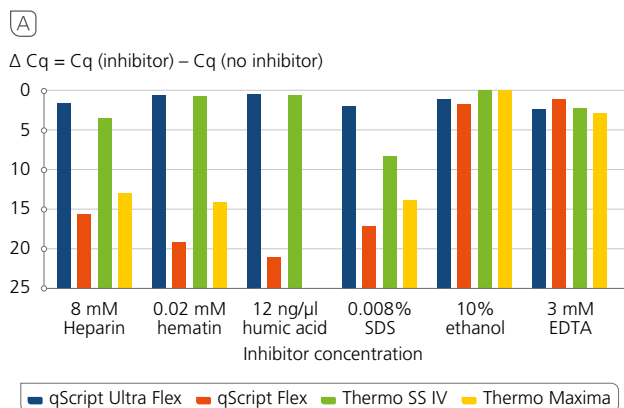
**Figure 3 Sensitivity Range.** Even with small quantities of highly degraded RNA extracted from FFPE liver tissue, qScript Ultra Flex Kit maintains linearity in cDNA synthesis over a range of RNA input and allows for a reproducible low limit of detection.



**Figure 4 Dynamic Range.** In an oligo(dT)-primed, first strand cDNA synthesis reaction series, qScript Ultra Flex Kit enables a reproducible low limit of detection with a broad linear dynamic range over eight orders of magnitude.

## Inhibitor Resistance

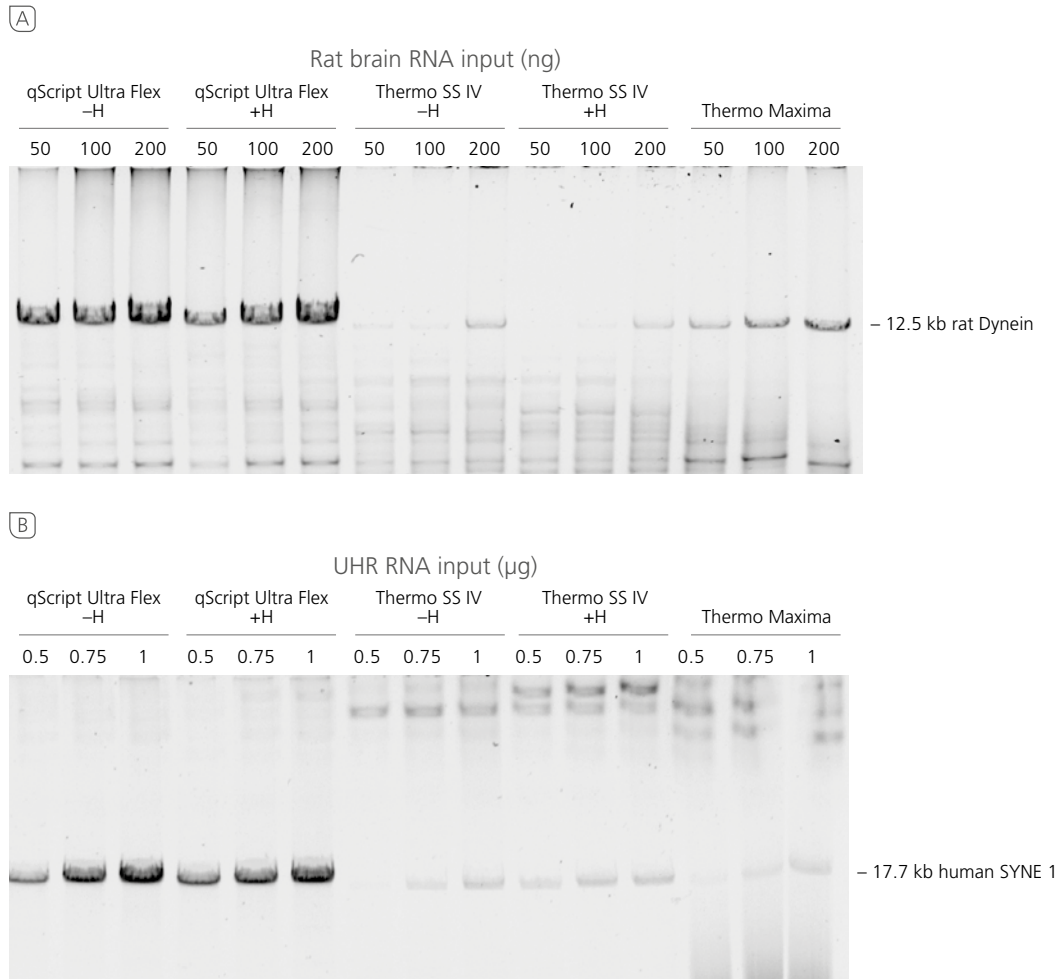
qScript Ultra Flex Kit yields high levels of cDNA, even in the presence of a wide range of inhibitors, which may be carried over due to RNA isolation from difficult or challenging sample matrices.



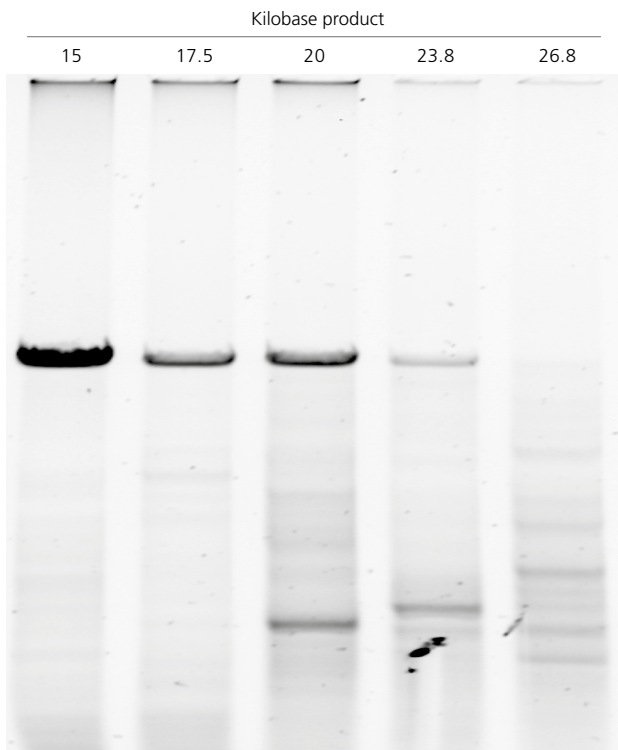
**Figure 5 Inhibitor Resistance Comparison.** The robust, engineered qScript Ultra reverse transcriptase is tolerant to many common reaction inhibitors. In standard random-primed first-strand cDNA synthesis reactions, the qScript Ultra Flex Kit maintains high yields of cDNA products in the presence of several inhibitors as indicated by the lack of large qPCR Cq shifts in the treated samples. Alternative kits were tested with and without inhibitors according to the manufacturer's recommended protocol.

## Longer Transcripts

qScript Ultra Flex Kit has the ability to reverse transcribe long RNAs up to 20 kb, which enables full-length cDNA synthesis and facilitates splice variant characterization, or applications such as transcriptome and RNA virus sequencing. When paired with Quantabio repliQa HiFi ToughMix, long transcripts of 12.5 kb and 17.7 kb were quickly reverse transcribed in 10 minutes followed by fast PCR amplification in 54 and 84 minutes, respectively. High yields of long product were generated using the qScript Ultra Flex Kit without a requirement for prior RNase H treatment. In contrast, PCR yields from other vendors were very low or absent using manufacturer recommended cDNA synthesis times.



**Figure 6 Long Range Capability.** cDNA synthesis reactions were carried out to 55°C for 10 minutes, and the cDNA used directly (-H) or treated with RNase H (+H). A portion of the products amplified using repliQa HiFi ToughMix. The Thermo Maxima reverse transcription mix has RNase H+ activity. **A** Various quantities of total RNA from rat brain were used for GSP-primed first-strand cDNA synthesis, then a 12.5 kb portion of the rat Dynein mRNA was amplified and analyzed. PCR amplification using repliQa HiFi ToughMix took only 54 minutes. **B** Various quantities of human total RNA were used for GSP-primed first-strand cDNA synthesis, then a 17.7 kb portion of the human SYNE1 mRNA was amplified and analyzed. PCR amplification using repliQa HiFi ToughMix took only 1 hour and 24 minutes.



**Figure 7** Increased Long Range Capability. One microgram of total human RNA was used for GSP-primed first-strand cDNA synthesis, then incrementally spaced 5'-forward primers were used with a common 3'-end reverse primer to amplify increasing fragment lengths of the human SYNE1 mRNA. Specific regions up to 23.8 kb were captured using a brief 10-minute cDNA synthesis step and 30 cycles of PCR with repliQa HiFi ToughMix.

## ORDER INFO

### Product Name

qScript Ultra Flex Kit - 25  
qScript Ultra Flex Kit - 100

### Quantabio Catalog Number

95215-025  
95215-100

### Size

25 rxns  
100 rxns

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