

AccuScript™ High Fidelity Reverse Transcriptase Products

Highest Accuracy RT Produces Premium cDNA

- + Unique, high fidelity reverse transcriptase synthesizes cDNA that contains 3- to 6-fold fewer errors than SuperScript® II and SuperScript® III RT
- + RNase H deficiency ensures high yields of full length cDNA—from 1 to 20 kb
- + Most accurate reverse transcriptase delivers premium cDNA
- + Multiple, convenient and complete kit formats offer flexibility for your application need
- + New enhanced stringent quality control procedures ensure high yields of premium cDNA

OUR ACCUSCRIPT™ REVERSE TRANSCRIPTASE (RT) DELIVERS THE BEST REVERSE TRANSCRIPTION ACCURACY DURING FIRST-STRAND cDNA SYNTHESIS. IT IS THE IDEAL CHOICE FOR ANY APPLICATION THAT REQUIRES PREMIUM ERROR-FREE cDNA.

Reverse transcriptases exhibit significantly higher error rates than other known DNA polymerases, introducing errors at frequencies of one per 1,500 to 30,000 nucleotides during cDNA synthesis¹ and are the major contributor of errors in reverse transcriptase PCR (RT-PCR), particularly when using ultra high-fidelity PCR enzymes (Table 1). These RT-introduced errors are then amplified exponentially during the PCR step and as a result, you must spend a significant amount of time and effort identifying and correcting errors after RT-PCR.

To solve the problem of low reverse transcription accuracy, we developed AccuScript™ High Fidelity Reverse Transcriptase*, which is engineered to deliver the highest transcription accuracy possible while promoting full-length cDNA synthesis, high yield and excellent RT-PCR performance.

We offer a complete line of AccuScript RT products:
AccuScript™ High Fidelity Reverse Transcriptase
AccuScript™ High Fidelity First-Strand cDNA Synthesis Kit
AccuScript™ High Fidelity RT-PCR Kit

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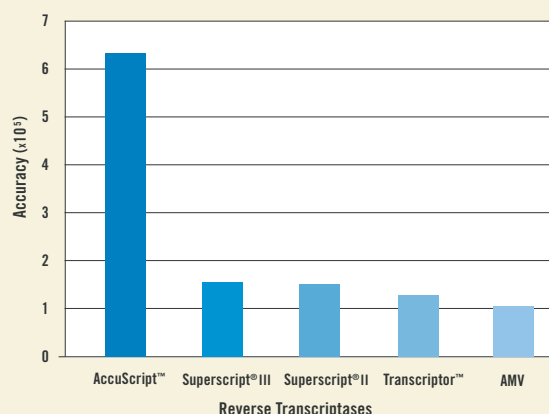


Figure 1
AccuScript™ Reverse Transcriptase Has the Highest Accuracy Rate.

AccuScript™ reverse transcriptase delivers greater than 3-fold higher accuracy than other RTs. Accuracy is defined as 1/error rate.

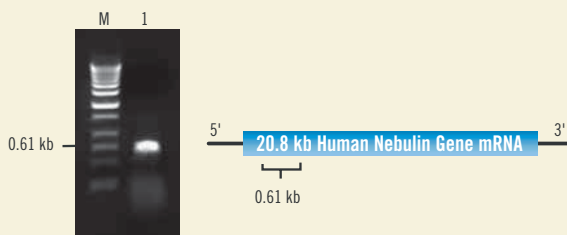


Figure 2
Full-Length cDNA up to 20kb and Great RT-PCR Yields.

A 0.61 kb region corresponding to the 5' end of the 20.8 kb human nebulin gene was successfully amplified, indicating the complete reverse transcription of the human nebulin gene.

AccuScript™ High Fidelity Reverse Transcriptase Products

This reverse transcriptase is engineered to deliver the highest transcription accuracy while promoting full-length cDNA synthesis, yield, and RT-PCR performance.

- + Accurate reverse transcription of first strand cDNA for use in PCR and sequence validation, particularly for viral RNA species identification and characterization
- + Generate full length cDNA synthesis for gene cloning, sequencing and protein expression experiments
- + Incorporate regular and amino allyl modified nucleotides during cDNA synthesis for use in microarrays and other labeling experiments

Most Accurate Reverse Transcriptase

Reverse transcriptases (RT) exhibit significantly higher error rates than other known DNA polymerases introducing errors at frequencies of one per 1,500 to 30,000 nucleotides during cDNA synthesis¹. To solve the problem of low accuracy, Stratagene introduces the new AccuScript reverse transcriptase, which is an RNase H-deficient Moloney murine leukemia virus reverse transcriptase (MMLV RT) we engineered to deliver the highest transcription accuracy while promoting cDNA synthesis length, yield and RT-PCR performance. As shown, AccuScript RT provides over 3.7-fold more accurate reverse transcription than other RTs such as SuperScript™ II and SuperScript™ III RTs and is 4.7-fold and 6.6-fold more accurate than Transcriptor™ RT and AMV RT, respectively (Figure 1). The development of AccuScript RT represents a significant advancement in cDNA synthesis accuracy.

RNase H-Free Means Longer cDNA

Both MMLV and AMV RT possess substantial RNase H activity that degrades RNA molecules and limits full-length cDNA synthesis and high yield. RT mutants lacking RNase H activity provide superior performance compared to MMLV and other RNase H+ RTs. To ensure maximum yields, AccuScript RT was derived from an RNase H deficient MMLV RT mutant. As an example of its full-length cDNA synthesis ability, we have demonstrated that AccuScript RT can synthesize cDNAs up to 20 kb in length (Figure 2). Also, AccuScript RT is stringently quality controlled to ensure that it is nuclease-free, for high cDNA yields.

Stringent Quality Control Ensures High Yields of Premium cDNA

We employ new, enhanced, and the most stringent quality control assays and procedures during the manufacturing process. These new specifications ensure that AccuScript RT is virtually nuclease-free, which allows you to generate premium quality cDNA using any of our AccuScript RT products. Compared to a leading competitor, our AccuScript RT has lower non-specific RNase activities and has virtually non-existent exonuclease activity (Figure 3). This ensures that during the first-strand cDNA synthesis reaction, your RNA will remain undegraded while it is copied accurately and efficiently, delivering the best quality cDNA.

Best Overall RT-PCR Accuracy

In the past, PCR enzymes such as *Taq* and *Taq*-based enzyme blends, introduced the majority of errors during RT-PCR amplification. However, with higher fidelity PCR enzymes such as *PfuUltra*™ High Fidelity DNA polymerase, the reverse transcriptase is the major contributor of errors. Using our fidelity data from the first-strand cDNA synthesis reaction with previously published PCR enzyme error rates, we predicted the percentage of clones containing errors after RT-PCR amplification (Table 1). For example, when 1 kb is reverse transcribed with SuperScript II RT and amplified with Platinum® *Taq* DNA Polymerase High Fidelity, the percentage of clones predicted to contain errors is 18%—the majority of these introduced via the PCR enzyme. In contrast, using AccuScript RT, the number of errors is 3.7-fold lower compared to SuperScript II RT and this significantly lowers the overall RT-PCR error rate. Furthermore, using *PfuUltra* DNA Polymerase to PCR-amplify the AccuScript RT-generated cDNA, fewer than 2.5% of the clones contain errors. This is seven times lower compared to using SuperScript RT with Platinum *Taq* DNA polymerase.

Multiple Kit Formats Deliver Convenience and Flexibility

AccuScript high fidelity RT products come in a range of formats to suit your every application. We offer the stand-alone reverse transcriptase enzyme shipped with its optimized buffer, allowing you maximum flexibility for your first-strand reaction set up and choice of downstream application. We also offer a complete first-strand cDNA synthesis kit that includes all of the necessary reagents for first-strand cDNA synthesis, saving you time. For maximum convenience and the highest fidelity RT-PCR, we also offer a complete two-tube RT-PCR kit. This kit contains AccuScript RT, our *PfuUltra* DNA polymerase, and all the necessary components for first-strand cDNA synthesis and PCR amplification, making this the most convenient method for delivering the highest accuracy RT-PCR.

Enzymes		Error Rate ($\times 10^{-6} \pm \text{SD}$)		Predicted Percent Mutant Clones
RT	PCR Enzymes	RT ¹	PCR	RT + PCR
AccuScript™ RT	<i>PfuUltra</i> ™ DNA Polymerase	16.1 ± 3.9	0.43 ± 0.04	2.5%
AccuScript™ RT	PicoMaxx™ DNA Polymerase	16.1 ± 3.9	4.0 ± 1.3	9.6%
SuperScript® RT	Platinum® <i>Taq</i> HiFi DNA Polymerase	64.1 ± 5.9	5.8 ± 0.3	18%

Table 1

Accuracy of RT-PCR Is Affected by the Fidelity of Both RT and PCR Enzymes

In this table, we illustrate a situation where an RNA template is reverse transcribed with AccuScript™ RT or SuperScript® II RT and then a 1-kb portion is PCR amplified for 20 duplications using different PCR enzymes. We predict mutation frequencies using the equation $\text{MF} = \text{ER} \times \text{bp} \times \text{d}$, where MF is the mutation frequency, ER is the error rate, bp is the length of the target, and d is the number of template doublings (10⁶-fold amplification equals 20 doublings). Using *PfuUltra*™ DNA polymerase, the percentage of 1 kb cDNAs that are expected to contain errors is 2.5%, where the contribution from the RT is 1.6% ($1.6 \times 10^{-5}/\text{base} \times 1000 \text{ bases}$), and the contribution from *PfuUltra* enzyme is 0.9%. In contrast, if the same RT-PCR reaction was carried out with SuperScript RT II and a *Taq* DNA polymerase blend, 6.4% of 1-kb cDNAs are expected to contain RT-generated errors, and an additional 11.6% of the final clones would contain Platinum® *Taq* HiFi polymerase generated mutations. The overall MF in this case would be almost 7x higher, at 18%.

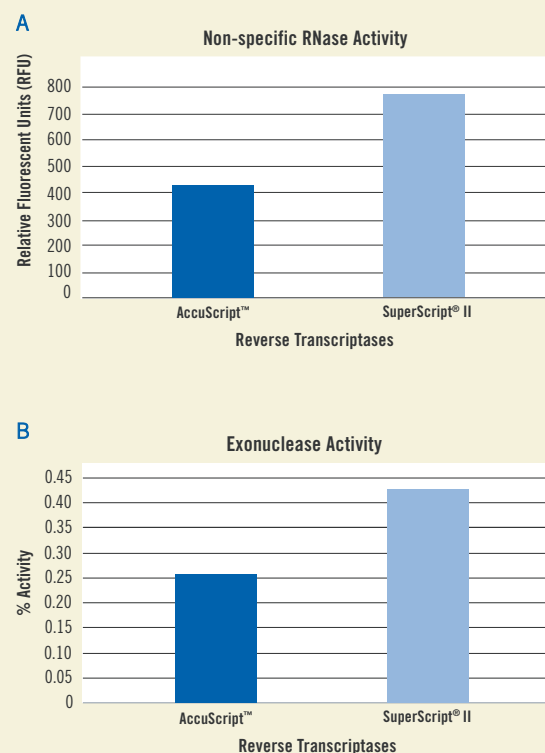


Figure 3

Stringent Quality Control Produces Nuclease-Free Enzyme

Shown here are two examples where AccuScript™ RT has substantially lower activities of non-specific RNase activity (A) and exonuclease activity (B) than that of SuperScript® II RT, which is considered to be high quality.

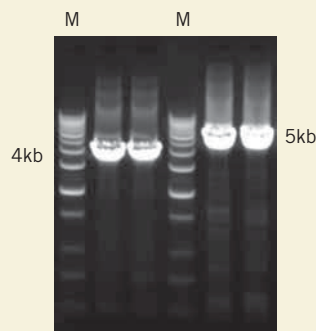


Figure 4
High-Yield RT-PCR Amplification With the PicoMaxx™ Enzyme

We performed duplicate two-step RT-PCR reactions using our AccuScript™ first-strand cDNA synthesis system with oligo dT primers in combination with PicoMaxx™ High Fidelity PCR System. This shows amplification of 4 kb and 5.1 kb fragments from the mouse complement gene.

M= Marker lane

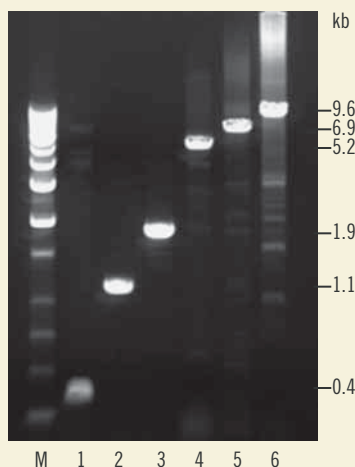


Figure 5
AccuScript™ System Maximizes Fidelity and Yield
AccuScript™ RT-PCR kit delivers high yields across a wide range of amplicon sizes.

AccuScript™ High Fidelity First Strand cDNA Synthesis Kit

- + Convenient kit format offers flexible oligo dT or random priming options
- + Complete system and streamlined protocol makes first-strand reaction set-up easy and fast
- + Formatted for high-yield cDNA production and flexibility in PCR enzyme selection depending on downstream application

Our AccuScript high fidelity first strand cDNA synthesis kit** helps you synthesize high-quality cDNA that is 3- to 6-fold more accurate than the kit you currently use. This high-quality cDNA can be used in downstream applications such as PCR amplification, cDNA library construction, and real-time PCR quantification. The kit is formatted specifically to produce a lot of cDNA and therefore accommodate a range of RNA amounts (pg to µg). It includes both random and oligo-dT primers depending on your preference or application. We also include high-quality, high-fidelity grade first-strand reagents to ensure your RT-PCR success. This kit uses a very streamlined protocol, which makes it easy and fast to set up multiple cDNA reactions, saving you valuable research time. For two-step RT-PCR, we recommend our *PfuUltra*™ DNA polymerase or PicoMaxx™ High Fidelity PCR System†,Ω. Figure 4 is a data set showing high yield amplification for 4-kb and 5-kb fragments using our PicoMaxx PCR system. High yield is paramount for successful gene cloning for protein expression studies.

AccuScript™ High Fidelity RT-PCR Kit

- + Excellent RT-PCR yields of high-quality cDNA, especially from long transcripts up to 9.6 kb
- + Allows you to create cDNA archive for precious RNA samples
- + Two-tube system ideally suited for cloning, expression, and sequencing

The AccuScript RT-PCR kit***,Ω product is the most convenient way for you to achieve the highest fidelity RT-PCR. This kit combines our AccuScript RT and ultra-high fidelity *PfuUltra* DNA polymerase for an unmatched winning fidelity combination. If you combine the AccuScript RT with lower fidelity PCR enzymes, you will lose the added benefit achieved with a high-fidelity RT because the PCR enzyme is the most significant contributor to overall RT-PCR fidelity (Table 1). When using AccuScript RT on a variety of RT-PCR targets, particularly longer ones, not only will you observe high accuracy but also high yield, even up to 9.6 kb (Figure 5). Using as little as 100 pg of total RNA in RT-PCR gives you the sensitivity needed to detect low and high abundant messages with smaller starting sample sizes.

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AccuScript™ Reverse Transcriptase & Related PCR Enzymes

AccuScript™ High Fidelity Reverse Transcriptase	50 reactions	600089
	200 reactions	600090
AccuScript™ High Fidelity First-Strand cDNA Synthesis Kit	50 reactions	200820
AccuScript™ High Fidelity RT-PCR Kit	50 reactions	600180
(includes <i>PfuUltra</i> ™ High Fidelity DNA Polymerase)		
<i>PfuUltra</i> ™ High-Fidelity DNA Polymerase	100 reactions	600380
PicoMaxx™ High Fidelity PCR System	100 reactions	600420

Reference

1. Roberts, J.D., Bebenick, K., Kunkel, T.A. The Accuracy of Reverse Transcriptase from HIV-1. *Science* 1988 (242) 1171-1173.

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SuperScript® II, SuperScript®, and Platinum® III are registered trademarks of Invitrogen.

Transcriptor™ is a trademark of Roche.

* Patents pending.

** Patents pending. Purchase of this PCR-related product does not convey any rights under the PCR patents owned by Roche Molecular Systems. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

†,Ω U.S. Patent Nos. 6,734,293, 6,489,150, 6,444,428, 6,379,553, 6,333,165, 6,183,997, 5,948,663, 5,866,395, 5,545,552 and patents pending.

*** Ω Patents pending. Purchase of these products is accompanied by a license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

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