Technically Speaking

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M-MLV Reverse Transcriptase, RNase H Minus

During cDNA synthesis, the RNase H activity of reverse transcriptases can interfere with the enzymes' ability to generate full-length cDNA from long RNA templates. Use of an RNase H minus reverse transcriptase (such as engineered forms M-MLV RT) increases the percentage of full-length cDNA synthesized and is recommended when generating full-length cDNA libraries.

For what applications are reverse transcriptases commonly used?

A reverse transcriptase (RT) is a type of DNA polymerase that catalyzes the synthesis of a complementary DNA (cDNA) from an RNA template (1–3). The ability of reverse transcriptases to make a cDNA copy of RNA is used in many different molecular biology applications including production of cDNA libraries, RT-PCR[a], primer extension and RNA sequencing.

What are the most commonly used RTs?

The two most commonly used reverse transcriptases are the retroviral enzymes AMV RT (Cat.# M5101), purified from Avian Myeloblastosis Virus (4,5), and M-MLV RT, purified from an *E. coli* strain expressing a recombinant clone of Moloney Murine Leukemia Virus RT (6,7).

What enzymatic activities do RTs possess?

Both AMV and M-MLV RT possess two major activities: an RNA-dependent DNA polymerase activity and an RNase H activity. The DNA polymerase activity is essential for all applications requiring cDNA synthesis. However, the RNase H activity is undesirable and interferes with the enzyme's ability to produce full-length cDNA. Note that reverse transcriptases also have a DNA-dependent DNA polymerase activity on DNA templates and RNA:DNA hybrids. However, this activity is not significant and is generally not exploited for molecular biology applications.

What is RNase H activity?

RNase H activity is an important consideration when choosing the most suitable reverse transcriptase for cDNA synthesis. Like the activity of *E. coli* RNase H, the RNase activity of reverse transcriptases degrades the RNA strand of an RNA:DNA hybrid. When cDNA is synthesized, RNA:DNA hybrids are generated, which can act as substrates for the RNase H activity of reverse transcriptases. The effect of the RNase H activity on cDNA synthesis is two-fold; both the total yield and the percentage of full-length transcripts are adversely affected. Reverse transcription is initiated from a DNA primer hybridized to a specific sequence in the RNA template, often the poly(A)+ tail. The primer:RNA hybrid not only acts as a priming site for polymerization but also as a substrate for the RNase H activity of the reverse transcriptase. The yield of cDNA synthesized is affected by the extent that the RNase H activity of the reverse transcriptase destroys the RNA:DNA hybrid before polymerization initiates. In some applications, this can increase the minimum amount of RNA required to detect cDNA synthesis. In addition, the RNase H activity of the enzyme can cleave the RNA strand near the site of DNA polymerization. If this occurs, the uncopied portion of the RNA molecule can dissociate from the transcriptional complex and cDNA synthesis stops. The longer the RNA molecule is, the more likely this is to occur. The net result is that long RNA molecules (>5kb) are less likely to be fully copied into cDNA when the RNase H activity of the reverse transcriptase is active.

How can I eliminate the RNase H activity of a RT?

The DNA polymerase activity of a reverse transcriptase is not dependent on the concomitant RNase H activity. As such, the deleterious effects of the RNase H activity on cDNA synthesis can be eliminated by engineering a reverse transcriptase lacking RNase H activity. This can be achieved by introducing a mutation in the RNase H domain of the protein. These reverse transcriptases are referred to as RNase H minus. Elimination of the RNase H activity results in more efficient initiation of reverse transcription, as the primer:RNA hybrids are not degraded. Elimination also results in more efficient synthesis of full-length cDNAs, since synthesis is not prone to early termination as described above.

What types of RNase H minus RTs are available from Promega?

Two types of RNase H minus reverse transcriptases are available from Promega: M-MLV Reverse Transcriptase, RNase H Minus (Cat.# M5301), Deletion Mutant; and M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant (Cat.# M3682). The "Point Mutant" form has been rendered RNase H minus by the introduction of a point mutation in the RNase H domain of the enzyme, and it is functionally equivalent to SuperScript™ II. The "Deletion Mutant" form is similar to the Point Mutant form, but the RNase H activity has been eliminated by a deletion mutation. M-MLV Reverse Transcriptase, RNase H Minus, is functionally equivalent to SuperScript™.

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What is the functional difference between the deletion and point mutant forms of M-MLV RT, RNase H Minus?

For most applications, either RNase H Minus form of M-MLV RT can be used. One difference we have observed is that the Point Mutant enzyme appears to have greater thermostability than the Deletion Mutant. In cDNA synthesis reactions using a 1.2kb template, we have observed full-length cDNA synthesis at temperatures up to 55°C using the M-MLV RT, RNase H Minus, Point Mutant. For the same template, full-length cDNA can be synthesized at temperatures up to 50°C using M-MLV RT, RNase H Minus, Deletion Mutant. Please call (1-800-356-9526, or 1-608-274-4330 outside the U.S.) or e-mail (*techserv@promega.com*) Promega Technical Services for more information on using these enzymes at elevated temperatures.

What if I am currently using one of the SuperScript™ enzymes?

For SuperScript™ II, we recommend that you substitute with M-MLV RT, RNase H Minus, Point Mutant (Cat.# M3682); for SuperScript™, substitute with M-MLV RT, RNase H Minus (Cat.# M5301). The enzymes can be used unit-for-unit in place of either SuperScript™ or SuperScript™ II enzymes in your applications.

Which applications benefit most from using an RNase H minus RT?

RNase H minus reverse transcriptase is the enzyme of choice for generating cDNA libraries. The ability of the enzyme to generate full-length cDNA from long templates means that the cDNA library will more accurately reflect the RNA population—that is, short and long transcripts—used to make the library. However, since the main advantage of using an RNase H minus reverse transcriptase is the ability of the enzyme to generate long cDNAs, an RNase H minus RT may not improve results obtained in RT-PCR or primer extension where the required cDNA is usually short. If a long cDNA (>5kb) is required for these applications, then we recommend using an RNase H minus RT.

In addition to the length of the RNA template, areas of complex secondary structure in the RNA template can affect the ability of a reverse transcriptase to generate full-length cDNA. Performing the cDNA synthesis reaction at elevated temperatures helps by relaxing the areas containing problematic secondary structure. In situations where secondary structure in the RNA template may be impeding synthesis of full-length cDNA and where performing the cDNA synthesis reaction at an elevated temperature is desired, use of M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant, is recommended. Both of these enzymes can be used at elevated temperatures.

References

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6. Roth, M.J., Tanese, N. and Goff, S.P. (1985) *J. Biol. Chem.* **260**, 9326.
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Ordering Information

Product	Size	Cat.#
M-MLV Reverse Transcriptase, RNase H Minus	10,000u	M5301
M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant	10,000u	M3682
	50,000u	M3683

Related Product	Size	Cat.#
M-MLV Reverse Transcriptase,	10,000u	M1701
RNase (H+)	50,000u	M1705
M-MLV Reverse Transcriptase Buffer Pack	2 × 1ml	M5313
AMV Reverse Transcriptase	300u	M5101
	1,000u	M5108
AMV Reverse Transcriptase, High Concentration	600u	M9004

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[a]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.