

## Frequently Asked Questions

### **Taq DNA Polymerase**

Catalog No. 11630-004	400 units
Catalog No. 11630-020	2,000 units
Catalog No. 11630-040	4,000 units

Q: How should I set up a PCR reaction using *Taq* DNA Polymerase?

A: The general guidelines are:

0.5-2.0 units *Taq* DNA Polymerase  
200  $\mu$ M each dNTP  
0.2-0.5  $\mu$ M each primer  
2-50 pg plasmid or 50-500 ng genomic template  
1 X PCR Buffer  
Denature at 94°C  
Extend 1 minute/kb

Q: Why is the product a smear when visualized on an agarose gel?

A: *Taq* DNA Polymerase has a half-life of 45 minutes at 94°C, therefore conditions for making long products (>5 kb) can degrade the polymerase. In this case, increasing the amount of *Taq* DNA Polymerase in the reaction can help.

Q: Why is there no product when visualized on an agarose gel?

A: There can be several reasons for this:

- The DNA template is too long (see above).
- Primer concentration may be too low. It should be above 0.4  $\mu$ M.
- Try fresh nucleotides.

Q: What type of DNA ends result from a primer extension reaction or a PCR reaction using *Taq* DNA Polymerase?

A: *Taq* DNA Polymerase generates single base 3' adenine overhangs, which are suitable for use in TA cloning vectors. Ends can be blunted by purifying DNA after primer extension and treating with a proofreading polymerase such as DNA Polymerase I, Large (Klenow) Fragment, or T4 DNA Polymerase.

Q: Can *Taq* DNA Polymerase be used for nick translation?

A: Yes. The presence of a 5'-3' exonuclease activity makes *Taq* a suitable choice for nick translation.

Q: Will the 5'→3' exonuclease activity of *Taq* DNA Polymerase degrade primers?

A: No. The exonuclease will only degrade double-stranded DNA that it encounters while extending a DNA fragment. It will degrade a secondary primer if bound to the same strand (e.g, a mutagenesis primer).

Q: When should *Taq* DNA Polymerase be selected for use in PCR?

A: *Taq* is an excellent polymerase choice for routine primer extensions throughout a wide range of template sources. When cost per reaction and yield are priorities, *Taq* DNA Polymerase is the industry standard. It is limited by its relatively low thermostability (half-life of 45 minutes at 94°C) and lack of a proofreading exonuclease domain. These characteristics make it an excellent choice for SNP Genotyping but make it a poor choice for work on long (>5 kb) extensions, on templates with a high degree of secondary structure, or in applications that strive to minimize error incorporation.

Q: Is *Taq* DNA Polymerases supplied with dNTPs?

A: No, the dNTPs must be ordered separately.

## M-MuLV Reverse Transcriptase

Catalog No. 11650-010 10,000 units

Catalog No. 11650-050 50,000 units

Q: Can M-MuLV Reverse Transcriptase be used in other buffers?

A: Optimal activity is seen in its unique buffer supplied with the product.

Q: Does M-MuLV Reverse Transcriptase require DTT?

A: Yes. Citation: Gerard G. F., and D' Alessio J. M., Chapter 6 (73-93) From: Methods in Molecular Biology, Vol.16: Enzymes of Molecular Biology Edited by: M. M. Burell 1993 Humana Press Inc. Totowa, NJ.

**Q:** How can the length of the product generated by M-MuLV Reverse Transcriptase be increased?

**A:** Incubation at 42°C can relax some RNA secondary structure allowing M-MuLV Reverse Transcriptase to produce longer products.

**Q:** What are some of the reasons for M-MuLV Reverse Transcriptase reaction failure?

**A:** SDS, DMSO, high salt, or EDTA in the nucleic acid template preparation can inhibit the reaction. M-MuLV Reverse Transcriptase is especially sensitive to the chelation effects of EDTA, since the Mg<sup>2+</sup> concentration of 3 mM is optimal. Other molecular biology techniques typically use 10 mM Mg<sup>2+</sup> so they are more able to tolerate traces of EDTA.

**Q:** Is M-MuLV Reverse Transcriptase active at temperatures higher than 42°C?

**A:** For specific difficult templates (e.g, with high GC content or significant secondary structure) a higher reaction temperature may be used but the overall yield of the DNA may be decreased. We have successfully copied 6 kb messages by reverse transcription at 55°C.

**Q:** What do you recommend for Quantitative RT-PCR?

**A:** Marligen's First-Strand cDNA Synthesis System for Quantitative RT-PCR (Cat. No. 11801-100) has been designed for the highest efficiency conversion of RNA to cDNA and is fully optimized for quantitative real-time PCR applications. The specially formulated reaction buffer contains oligo dT and random hexamers and provides unbiased representation and equivalent cDNA abundance over a wide range of input RNA. The high efficiency conversion provides maximum sensitivity for quantification of low abundance RNAs and single-cell expression profiling. The patent-pending formulation captures both 3'-end and 5'-end cDNA sequence and comes in a convenient pre-mixed format that simplifies reaction assembly and generates improved precision and reproducibility in RT-PCR applications.

**Q:** Can DNA be used as a template for M-MuLV Reverse Transcriptase?

**A:** Yes, but the reaction is less efficient.

For further technical assistance please contact our Technical Service Department at [technical.support@marligen.com](mailto:technical.support@marligen.com) or (301)-874-4990.