Tetro Reverse Transcriptase

Shipping: On Dry/Blue Ice

Catalog numbers

BIO-65050 10,000 units

Batch No.: See vial

Concentration: 200 U/µL

Store at –20 °C

Storage and stability:
Tetro Reverse Transcriptase is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality Control:
The Tetro Reverse Transcriptase and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety Precautions:
Please refer to the material safety data sheet for further information.

Notes:
For research use only.

Description

Tetro Reverse Transcriptase is a highly sensitive, high stability M-MLV reverse transcriptase. Tetro Reverse Transcriptase is optimized for reverse transcription reactions using a wide range of total RNA amounts (5µg), such that long and low abundance mRNA can be detected by amplification after cDNA synthesis.

Tetro Reverse Transcriptase is suitable for first-strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR analysis of gene expression. Tetro Reverse Transcriptase can be used with total RNA, mRNA, *in-vitro* transcribed RNA or viral RNA.

Kit components

Reagent	10,000 units
Tetro Reverse Transcriptase	50 µL
5x Reaction Buffer	1.2 mL

Reaction Recommendations and Optimization

Template Quality

- Intact, high-quality RNA is essential for the reverse-transcription reaction.
- All reagents for use with RNA must be prepared using DEPC-treated water.
- The inclusion of an RNase Inhibitor can reduce template degradation and increase yield of PCR product (BIO-65027).
- Low-copy-number genes may require an increase in starting material.
- It is necessary to use a suitable RNA extraction reagent e.g., TRIsure™ (BIO-38032) or RNA Isolation Kit (BIO-52072).

Primer Design and Concentration

There are three methods for priming cDNA synthesis:

- **Oligo dT Primers**
  Oligo dT priming (BIO-38029) uses the poly-A tail found on the 3' end of most eukaryotic mRNAs. This ensures that the 3' end of mRNAs are represented, although long mRNAs can have their 5' ends under-represented in the subsequent cDNA pool. Use at 0.5 µM final concentration.

- **Random Hexamers**
  Random priming (BIO-38028) gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Random priming is unable to distinguish between mRNA and other RNA species present in the reaction. Use at 2.0 µM final concentration.

- **Gene Specific Primers (GSP)**
  Gene specific primers are designed to generate cDNA for a specific gene of interest. It is a widely used method for performing One-Step RT-PCR when only 1 gene is under investigation. It can be useful when RNA concentrations are low. Use at 0.4 µM final concentration.

A combination of Oligo dT and Random Hexamer primers can improve the reverse transcription efficiency of some mRNA templates.

Extension Temperature

- Efficient reverse-transcription can be achieved at temperatures of 37 °C to 45 °C for 30-60 min.
- The use of higher incubation temperatures up to 48 °C may increase the yield of cDNA synthesized in cases of complex RNA secondary structure. However, the yield of the majority of RNA molecules will be reduced.

Tetro Reverse Transcription Protocol

1. Vortex solutions and centrifuge briefly before use.
2. Prepare the priming premix on ice in an RNase-free reaction tube:

Total RNA (up to 5 µg) or mRNA (up to 0.5 µg)	*n* µL
Primer*: Oligo $(dT)_{18}$ (10 µM) *or* Random Hexamer (40 µM) *or* GSP (8 µM)	1 µL
10mM dNTP mix*	1 µL
5x RT Buffer	4 µL
RiboSafe RNase Inhibitor	1 µL
Tetro Reverse Transcriptase (200 U/ µL)	1 µL
DEPC-treated water	to 20 µL

3. Mix gently by pipetting.
4. Incubate samples at 45 °C for 30 min. If using random hexamers, incubate 10 min at 25 °C followed by 45 °C for 30 min.
5. Terminate reaction by incubating at 85 °C for 5 min, chill on ice.
6. Store reaction at –20 °C for long term storage, or proceed to PCR immediately.

This protocol is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

Website: www.bioline.com/ email: info@meridianlifescience.com

Troubleshooting

Problem	Possible Cause	Recommendation
No cDNA synthesis	RNA degraded	Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free. Use Ribosafe RNase inhibitor in the first-strand reaction (BIO-65027).
	RNA contained an RT inhibitor	The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step.
	Reaction temperature not optimal	Perform a temperature-gradient experiment ranging from 37-48 °C.
	Not enough starting RNA	Increase the amount of starting RNA, this can be an important factor when amplifying low-copy genes from total RNA.
	RNA had high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 48 °C (for short amplicons).
	Insufficient product	Increase reverse transcription step to 60 minutes
Poor Specificity in PCR	Non-specific annealing of primers to template	Use gene-specific primers rather than Oligo dT or random hexamers in RT reaction. Increase the annealing temperature in PCR. Check for presence of pseudogenes. Set up reactions on ice.
	Primer dimers	Redesign primers to prevent self-annealing.
	Genomic DNA contamination	Treat RNA with DNase I and re-purify. If possible, use intron-spanning primers in PCR.
Product in no-RTase control	Template contaminated with DNA	Treat samples with DNase I.

Technical Support:

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: tech@meridianlifescience.com

Product Citations:

1. To, K.W., *et al. Mol. Can. Res.* **9**, 516-527 (2011).
2. Comerford, I., *et al. Blood* **116(20)**, 4130-4140 (2010).
3. Corripio-Miyar, Y., *et al. Mol. Immunol.* **46(10)**, 2098-2106 (2009).
4. Chen, Y., *et al. Blood* **114(1)**, 40-48 (2009).
5. Le, H. K., *et al. Can. Immunol. Immunother.* **58(10)**, 1565-1576 (2009).

Associated products:

Product Name	Cat. No.
RiboSafe RNase Inhibitor	BIO-65027
TRIsure™	BIO-38032
ISOLATE II RNA Mini Kit	BIO-52072
Random Hexamer Primer	BIO-38028
Oligo (dT)18 Primer	BIO-38029
dNTP Mix (10 mM)	BIO-39053
Tetro cDNA Synthesis Kit	BIO-65043
SensiFAST™ SYBR No-ROX Kit	BIO-98002
Agarose, Molecular Grade	BIO-41026

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