

Tetro cDNA Synthesis Kit

Shipping: On Dry/Blue Ice Catalog numbers
Exp. Date: See vial BIO-65042 : 30 reactions
Batch No.: See vial BIO-65043 : 100 reactions



A Meridian Life Science® Company

Store at -20°C

Storage and stability:

Tetro cDNA Synthesis Kit is shipped on Dry/Blue Ice and should be stored at -20°C upon receipt. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Unit definitions:

Reverse Transcriptase: One unit catalyzes the incorporation of 1nmole of dTTP into acid-insoluble material in 10 minutes at 37°C in 50 mM Tris-HCl, pH 8.6, 40mM KCl, 1mM MnSO₄, 1mM DTT, and 0.5mM [3H]TTP, using 200µM oligo(dT)₁₂₋₁₈-primed poly(A)_n as template.
RNase Inhibitor: One unit inhibits 5ng of RNase A by 50%.

Safety precautions:

Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.



Signal word: WARNING

Description

The Tetro cDNA Synthesis Kit contains all the necessary components to generate cDNA from an RNA template. The generated cDNA is suitable for PCR with gene-specific primers or for other downstream applications. The kit contains MMLV Reverse Transcriptase and is suitable for first strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR amplification.

The Tetro cDNA Synthesis Kit is optimized for RT reactions using a wide range of total RNA amounts (100pg-2µg), such that long and low abundance cDNAs can be detected by amplification after cDNA synthesis. The Kit contains oligo (dT)₁₈ and random hexamer primers. The dNTPs included in the kit are manufactured by Bioline and are 99% pure.

Components

Product Name	30 reactions	100 reactions
5x RT Buffer	120µl	400µl
(200u/µl) Reverse Transcriptase	30µl	100µl
(10u/µl) RNase Inhibitor	30µl	100µl
dNTP Mix 10mM Total	30µl	100µl
Oligo (dT) ₁₈ Primer Mix	30µl	100µl
Random Hexamer Primer Mix	30µl	100µl
DEPC-treated Water	1.8ml	1.8ml

Reaction Recommendations and Optimization

- The use of RNase-free plasticware and tips is essential.
- We recommend using a final volume of 20µl.
- Prepare all reactions on ice.
- 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 cDNA Synthesis Kit Reaction Guidelines

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 (BIO-38030).
- The inclusion of an RNase Inhibitor protein can reduce template degradation and increase yield of PCR product.
- Low-copy-number genes may require an increase in starting material.
- Use a suitable RNA extraction reagent e.g. TRIsure™ (BIO-38032) or RNA Isolation Kit (BIO-52043).

Primer Design and Concentration

There are three methods for priming cDNA synthesis:

• Oligo dT Primers

Oligo dT priming 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.

• Random Hexamers

Random priming 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.

• 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 0.4µM/reaction).

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

Tetro cDNA Synthesis Kit 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 5µg) or mRNA (up to 5µg)	nµl
Primer: Oligo (dT) ₁₈ or Random Hexamer (or GSP)	1µl
10mM dNTP mix	1µl
5x RT Buffer	4µl
Ribosafe RNase Inhibitor	1µl
Tetro Reverse Transcriptase (200u/µl)	1µl
DEPC-treated water*	to 20µl

* Available separately (see Associated Products)

3. Mix gently by pipetting.
4. Incubate samples at 45°C for 30 min. If using random hexamers, incubate at 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.

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.
	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@bioline.com

Product Citations:

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Associated products:

Product Name	Cat. No.
SensiFAST SYBR No-ROX Kit	BIO-98002
ISOLATE RNA Mini Kit	BIO-52043
TRIsure™	BIO-38032
HyperLadder™ I	BIO-33025
DEPC-treated Water	BIO-38030
Agarose, Molecular Grade	BIO-41026

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