Tetro™ cDNA Synthesis Kit

Shipping: On Dry/Blue Ice

Catalog numbers

BIO-65042: 30 reactions

Batch No.: See vial

BIO-65043: 100 reactions

Store at –20 °C

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Storage and stability:
Tetro cDNA Synthesis Kit is shipped on dry/blue ice. All kit components should be stored at -20 °C upon receipt. Excessive freeze/thawing is not recommended. Thaw, mix, and briefly centrifuge each component before use.

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:
Tetro cDNA Synthesis Kit and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Unit definitions:
Reverse Transcriptase: One unit catalyzes the incorporation of 1nmole of dTTP into acid-insoluble material in 10 minutes at 37 ºC in 50 mM Tris-HCl, pH 8.6, 40 mM KCl, 1 mM $MnSO_4$, 1 mM DTT, and 0.5 mM [3H]TTP, using 200 µM 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

Notes:
For research or further manufacturing use only.

Trademarks:

Description

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.

Tetro cDNA Synthesis Kit is optimized for RT reactions using a wide range of total RNA amounts (5 µg), such that long and low abundance mRNA can be detected by amplification after cDNA synthesis. The kit contains oligo $(dT)_{18}$ and random hexamer primers.

Components

Product Name	30 reactions	100 reactions
5x RT Buffer	120 µL	1.2 mL
(200 U/ µL) Reverse Transcriptase	30 µL	100 µL
(10 U/ µL) RNase Inhibitor	30 µL	100 µL
dNTP Mix 10mM Total	30 µL	100 µL
Oligo $(dT)_{18}$ Primer Mix	30 µL	100 µL
Random Hexamer Primer Mix	30 µL	100 µL
DEPC-treated Water	1.8 mL	1.8 mL

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.
- 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 ISOLATE II RNA Mini Kit (BIO-52072).

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-qPCR when only 1 gene is under investigation. It can be useful when RNA concentrations are low (use at 0.4 µM/reaction).

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

Reaction Recommendations and Optimization

- The use of RNase-free plasticware and tips is essential.
- We recommend using a final volume of 20 µL.
- 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 Protocol

1. Vortex solutions and centrifuge briefly before use.
2. Prepare the priming mastermix 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 $(dT)_{18}$ *or* Random Hexamer (*or* GSP)	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 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.

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.
	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: mbi.tech@meridianlifescience.com

Associated products:

Product Name	Cat. No.
SensiFAST™ SYBR No-ROX Kit	BIO-98002
ISOLATE II RNA Mini Kit	BIO-52072
TRIsure™	BIO-38032
HyperLadder™ 1kb	BIO-33025
Agarose, Molecular Grade	BIO-41026

Product Citations:

1. Willis, A., *et al. Harmful Algae* **82** 19-25.(2019).
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3. Alkowni, R., *et al. J. Plant Pathol.* **1-5** (2019).
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6. Liying, C. *et al. Leukemia* **27(4):** 813-822 (2013).
7. Ahmadimoghaddam, D., *et al. Biol. Reprod.* **88(3):** 1-10 (2013).
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9. Bałkowiec-Iskra, E., *et al. Neurosci.* **180:** 322-333 (2011).
10. Szczepanek, K., *et al. J. Biol. Chem.* **286:** 29610-29620 (2011).

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