TetroScript Reverse Transcriptase

Shipping: On Dry/Blue Ice

Catalog numbers

BIO-65111: 10,000 units

BIO-65112: 4 x 10,000 units

Batch No.: See vial

Concentration: 200 U/μL

Store at –20 °C

Storage and stability:
TetroScript 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. 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:
TetroScript 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.

Trademark
SensiFAST is a trademark of Bioline Reagents Ltd.

Notes:
For research or further manufacturing use only.

Description

TetroScript Reverse Transcriptase has been developed to reduce RNase H activity and increase thermal stability. The enzyme can be used for cDNA synthesis at temperature up to 60°C, enabling melting of areas of secondary structure in RNA, improving cDNA yield and sensitivity from difficult RNA targets such viral genomes.

Kit components

Reagent	10,000 units	4 x 10,000 units
TetroScript Reverse Transcriptase	50 µL	4 x 50 µL
5x Reaction Buffer	1 mL	4 x 1 mL

Reaction Recommendations and Optimization

Template Quality

- 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., 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 (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-qPCR 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 50 °C for 30-60 min. Increase the reaction temperature to 55°C for gene-specific primer (GSP) and difficult templates or templates with high secondary structure. If required, reaction temperature can be increase up to 60 °C
- The use of higher incubation temperatures up to 60 °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.

TetroScript Reverse Transcription Protocol

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

Component	Volume
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
DEPC-treated water	to 10 µL

3. Incubate samples at 65°C for 5 min, then chill on ice for at least 1 min.
4. Prepare the reaction premix:

Component	Volume
5x Reaction Buffer	4 µL
RiboSafe RNase Inhibitor	1 µL
TetroScript Reverse Transcriptase (200 U/µL)	1 µL
DEPC-treated water	to 10 µL

5. Add 10 µL of the reaction premix to the priming premix and mix gently by pipetting.
6. Incubate samples at 50°C for 30 min. If using random hexamers, incubate 5 min at 25°C followed by 50°C for 30 - 60 min. Increase the reaction temperature to 55°C for gene-specific primer.
7. Terminate reaction by incubating at 70°C for 15 min, chill on ice.
8. Store reaction at –20°C for long term storage, or proceed to PCR immediately.

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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 50 – 60 °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 60 °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.

Associated products:

Product Name	Cat. No.
RiboSafe RNase Inhibitor	BIO-65027
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
SensiFAST™ Probe No-ROX Kit	BIO-86005
Agarose, Molecular Grade	BIO-41026

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.

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