DATA SHEET

cDNA Synthesis Kit

Shipping: On Dry/Blue Ice

Exp. Date: See vial

Batch No.: See vial

Concentration: see vial

Catalog numbers

BIO-65025: 30 Reactions

BIO-65026: 100 Reactions

Store at –20°C

Storage and stability:
The cDNA Synthesis Kit is shipped on Dry/Blue Ice upon receipt, all kit components should be stored at -20°C. Excessive freeze/thawing is not recommended. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Quality control:
Bioline operates under the ISO 9001 Management System.

Safety precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Notes:
This product insert is a declaration of analysis at the time of manufacture
Research Use Only

Features

- Generate high quality cDNA for any downstream application
- Highly suited to low concentrations of Total RNA ≥100pg
- Convenient, reliable, cost-effective
- Reverse transcribes RNA templates up to 9Kb
- Compatible with SensiMix reagents

Applications

- 1st strand cDNA synthesis for subsequent quantitative PCR
- Construction of cDNA libraries
- Two-step real-time PCR assays
- Generation of probes for hybridization
- Gene cloning

Description

The cDNA Synthesis Kit contains all necessary components to generate cDNA from a variety of RNA templates. The generated cDNA is suitable for real-time and end point PCR with gene-specific primers or for other downstream applications. The kit contains MMLV reverse transcriptase and is also suitable for first strand cDNA synthesis and cDNA library construction.

For the quantification of RNA transcripts, quantitative, real-time RT-PCR is the most sensitive and reliable method. The advantage of using the cDNA Synthesis Kit for two-step real-time PCR over using a one-step kit is better sensitivity and for maintaining archival cDNA.

The cDNA Synthesis Kit is optimized for reverse transcription reactions using a wide range of total RNA amounts (100pg-2µg), such that long and low abundance transcripts are represented after cDNA synthesis. The kit contains oligo $(dT)_{18}$ and random hexamer primers together with control RNA template. The oligo $(dT)_{18}$ and random hexamer primers can be used separately or in a mix (to overcome 5' and/ or 3' end bias associated with individual strategies).

Kit components

Reagent	30 Reactions	100 Reactions
5x RT Buffer	120µl	400µl
(200u/µl) Reverse Transcriptase	7.5µl	25µl
(10u/µl) Ribosafe RNase Inhibitor	30µl	100µl
dNTP Mix 10mM Total	30µl	100µl
Oligos $(dT)_{18}$ Primer Mix	30µl	100µl
Random Hexamer Primer Mix	30µl	100µl
Control RNA Template 1µg/µl (enough for 5 reactions)	5µl	5µl
DEPC-treated Water	1.8ml	1.8ml

cDNA Synthesis Kit Reaction Guidelines

Template Quality

- Intact, high-purity 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 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.

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 42°C for 30-60 min.
- The use of higher incubation temperatures up to 45°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.

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 5µg) or mRNA (up to 0.5µg)	*n*µl
Primer: Oligo $(dT)_{18}$ *or* Random Hexamer *or* (GSP)	1µl
10mM dNTP mix	1µl
DEPC-treated Water	up to 10µl

3. Incubate samples at 70°C for 5 min, then chill on ice for at least 1 min.
4. Prepare the reaction premix:

5x RT Buffer	4µl
Ribosafe RNase Inhibitor	1µl
Reverse Transcriptase (200u/µl)	0.25µl
DEPC-treated Water	to 10µl

5. Add 10µl of the reaction premix to the priming premix and mix gently by pipetting.
6. Incubate samples at 42°C for 30 min. If using random hexamers, incubate 10 min at 25°C followed by 45°C for 30 min.
7. Terminate reaction by incubating at 85°C for 5 min, chill on ice.
8. 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.

Two-Step real-time PCR

The product of the first strand cDNA synthesis can be used directly in real-time PCR reactions. The volume of cDNA to use in a single PCR is dependent upon the copy number of the target gene. We suggest using 100ng cDNA per reaction, however it may be necessary to vary this amount. Store reverse-transcription reactions on ice and proceed directly with real-time PCR, or for long-term storage, store reverse-transcription reactions at –20°C.

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.

Associated Products:

Product Name	Pack Size	Cat. No.
TriSure™	100ml	BIO-38032
ISOLATE RNA Mini Kit	50 reactions	BIO-52043
HyperLadder I	200 Lanes	BIO-33025
DEPC-Treated Water	100ml	BIO-38030
MyTaq HS Polymerase	250 units	BIO-21111
Tetro cDNA Synthesis Kit	100 reactions	BIO-65043
SensiMix SYBR No-ROX Kit	250 reactions	QT650-02

Troubleshooting guide

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 a reverse transcription 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 37-45°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 has high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the reverse transcription step, up to a maximum of 45°C (for short amplicons)
	Insufficient product	Increase reverse transcription step to 60 minutes
Poor Specificity	Non-specific annealing of primers to template	Use gene-specific primers rather than Oligo dT or random hexamers 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