



First Strand cDNA Synthesis Kit

Catalogue Numbers:

DNP-FS30 30 Reactions

Features

- Generate high quality cDNA for any downstream application
- Highly suited to low abundance total RNA up to 100pg
- Convenient, reliable, cost-effective

Applications

- 1st strand cDNA synthesis for quantitative PCR
- Construction of cDNA libraries
- 2-step RT-PCR assays
- Generation of probes for hybridization
- Gene cloning

Description

The First Strand 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 First Strand 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 together with control RNA template. The dNTPs included in the kit are 99% pure.

Kit components:

Component	30 Reactions
5x RT Buffer	120µl
(200u/µl) Reverse Transcriptase	30µl
(10u/µl) RNase Inhibitor	30µl
dNTP Mix 10mM Total	30µl
Oligos (dT) ₁₈ Primer Mix	30µl

First Strand cDNA Synthesis Kit Protocol

1. Prepare the following on ice:

(1µg) RNA	nµl
Oligo (dT) ₁₈ or Random Hexamer	1µl
10mM dNTP	1µl
DEPC-treated Water	up to 10µl

2. Incubate samples at 65°C for 10 minutes.

3. Place on ice for 2 minutes.

4. Prepare the following reaction mix:

	1 Reaction	10 Reactions
5x RT Buffer	4µl	40µl
RNase Inhibitor	1µl	10µl
Reverse Transcriptase (200u/µl)	1µl	10µl
DEPC-treated Water	to 10µl	to 100µl

5. Add 10µl of the above reaction mix to a tube containing the primed RNA.

6. Incubate samples at between 37-45°C for 30-60 minutes.

7. Terminate reaction by incubating at 70°C for 15 minutes, chill on ice.

These data are intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

Random Hexamer Primer Mix	30µl
Control RNA Template 1µg/µl (enough for 5 reactions)	5µl
DEPC-treated Water	1.2ml

Product Specifications

Batch details:

Batch No: See vial
Units per vial: See vial
Concentration: See vial

Storage Conditions:

The First Strand cDNA Synthesis Kit can be stored for up to 6 months at -20°C.

Shipping Conditions:

On Dry Ice or Blue Ice

The First Strand cDNA Synthesis Kit contains enough reagents for either 30 or 100 single-strand reactions.

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.



Xn: HARMFUL
Xi: IRRITANT

Notes

1. Available in USA only.
2. This product insert is a declaration of analysis at the time of manufacture.
3. Research Use Only.

First Strand 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

Primer Design and Concentration

There are three methods for priming cDNA synthesis:

- **Oligo dT**
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 (use at 50 pmoles/reaction).
- **Random Primers**
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 (Use at 50-250ng/reaction).
- **Gene Specific Primers**
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 10-20pmol/reaction).

For most applications Oligo dT priming is recommended.

Reaction Recommendations

- The use of RNase-free plasticware and tips is essential
- We recommend using a final volume of 50µl.
- Prepare all reactions on ice.
- Efficient reverse-transcription can be achieved at temperatures of 37°C to 45°C for 15-30 minutes. We recommend that initial reverse-transcription steps are carried out for 30 minutes at 42°C
- The use of higher incubation temperatures up to 50°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.

First Strand cDNA Synthesis Kit Troubleshooting Guide

Observation	Possible Cause	Recommended solution(s)
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.
	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).
Poor Specificity	Target not expressed in tissue analysed:	Try a different target of tissue.
	Non-specific annealing of primers to template:	Use gene-specific primers rather than Oligo dT or random hexamers. Increase the annealing temperature. Increase the T _m of the primers. Check for presence of pseudogenes. Set up reactions on ice.
	Primer dimers:	Redesign primers to prevent self-annealing.
Product in no-RTase control	Genomic DNA contamination:	Try a different target of tissue.
	Template contaminated with DNA:	Treat samples with DNase I.