

One-Step RT-PCR Kit

Catalog Number: LSI-KIT-101

Simple to use RT-PCR Kit made by Cosmo Bio USA.

Kit Components:

One-Step RT PCR Kit	100 Reactions
Enzyme Mix	200uL
2X Reaction Buffer	2x1.25mL
RNase Inhibitor (10u/uL)	100uL
50mM MgCl ₂	1.2mL
DEPC treated water	1.2mL

Protocol:

COMPONENT	VOLUME (μl)	FINAL CONCENTRATION
2x One-Step RT-PCR Buffer (supplied)	25	1x
One-Step Enzyme Mix (supplied)	2	-
Forward Primer (5μM)	2	200nM
Reverse Primer (5μM)	2	200nM
RNA Sample	1-10	User-determined (100pg-2μg recommended)
RNase Inhibitor (supplied)	1	10 Units
MgCl ₂ (supplied)	2x Reaction Buffer contains 3mM MgCl ₂ . However additional Mg ²⁺ may be required (see reaction guidelines)	1.5mM (Unless adjusted by the user)
DEPC-Treated Water (supplied)	Up to final volume of 50μl	-
Total Volume		50μl

1 cycle of:

Temperature	Duration	Comments
37-45°C	15-30 minutes	We recommend that initial reverse-transcription steps are carried out for 30 minutes at 42°C (see reaction guidelines)
95°C	10 minutes	To denature RT enzyme and activate DNA Polymerase

Followed by 40 cycles of:

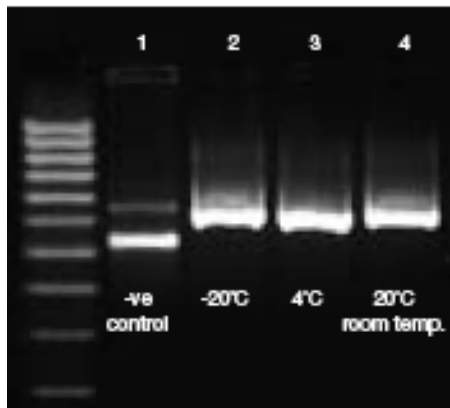
Temperature	Duration	Comments
95°C	30 seconds	Template denaturation
50-60°C	30 seconds	Primer annealing (actual temperature determined by primer sequence, see guidelines)
72°C	15-30 seconds per kilobase	Extension step

This kit is for research use only, not for diagnostic and clinical uses.

Technical information

Ultra-stable

1 Week Stability Assay

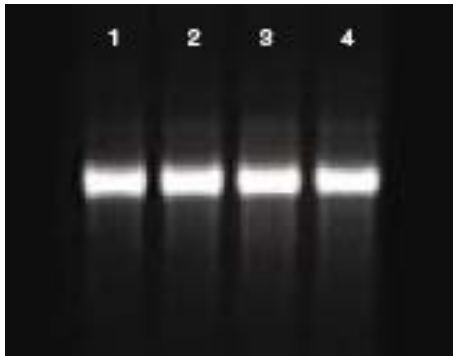


Reverse Transcription experiments using an in vitro RNA transcript and the RT enzyme. To demonstrate the temperature stability of the kit, a 500bp fragment was amplified using the kit which had been subjected to one week of room temperature, +4°C and -20°C respectively.

1. Control-no reverse transcriptase
2. -20°C for one week
3. +4°C for one week
4. Room temp for one week

Note: lanes 2,3 and 4 show a band corresponding to the RNA:DNA hybrid, as opposed to lane 1 where only RNA is observed.

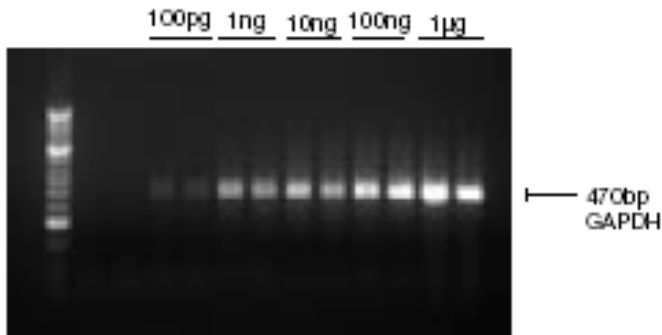
No RNase Activity



2 μ g of 1.5Kb poly(A)-tailed RNA were incubated with 200u of the Enzyme at 37°C for one hour, followed by phenol extraction and then subjected to formaldehyde gel analysis.

1. RNA fragment with RNase-free water kept on ice for one hour
2. RNA fragment with RNase-free water incubated at 37°C for one hour
3. RNA fragment in the reaction buffer incubated at 37°C for one hour
4. RNA fragment in the reaction mixture incubated at 37°C for one hour

Sensitivity of the One-Step RT-PCR Kit



RT-PCR was carried out on HeLa cell total RNA, at the indicated concentrations, using gene-specific primers. Reverse transcription was performed at 49°C for 30 minutes and then followed by 40 cycles of PCR. Samples were analyzed on a 2% agarose gel.