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One Step RT-PCR® Kit

Catalogue Numbers:

DNP-RT25 25 Reactions

Features

- Simple to use
- One-step set-up
- 2x RT-PCR Mix optimized for as little as 100pg Total RNA
- Contains ultra-pure dNTP's

Applications

- Quantitative PCR
- Gene expression analysis
- Gene cloning

Description

The One-Step RT-PCR Kit has been designed for highly sensitive one-step RT-PCR reactions using any RNA template. The Kit employs an enzyme formulation, which includes our widely used hot-start DNA polymerase and MMLV reverse transcriptase. The One-Step RT-PCR Kit provides highly specific reverse transcription and PCR in a single tube, using gene-specific primers on either total RNA or mRNA. The kit is provided with RNase inhibitor to protect template RNA from degradation. The proprietary buffer is highly optimised and balanced, leading to outstanding results. The Kit is ideal for the synthesis of double-stranded cDNA products for subsequent cloning, sequencing, expression, or transcription analysis. The Kit can be used with starting amounts of RNA template from 100pg to 2µg. After cDNA synthesis has been performed, the reaction is heated to 95°C for 10 minutes to inactivate the reverse transcriptase, and simultaneously to activate the hot-start DNA polymerase IMMOLASE (included). IMMOLASE improves specificity by eliminating the presence of non-specifics, primer-dimers, and mis-primed products.

Kit components:

Enzyme Mix
2x One-Step RT-PCR
Reaction Buffer
RNase Inhibitor (10u/µl)
50mM MgCl₂
DEPC-treated Water

Product Specifications

Batch details:

Batch No: See vial

Units per vial: See vial

Concentration: See vial

Storage Conditions: The One-Step RT-PCR Kit can be stored for 6 months at -20°C, in a constant-temperature freezer.

Shipping Conditions: On Dry Ice or Blue Ice



Xn: Harmful
XI: Irritant

Components:

The One-Step RT-PCR Kit is available in three pack sizes and contains sufficient reagents for 10, 25 or 100 reactions.

Associated Products:	Pack Size
dNTP Set	4 x 25µmol
HyperLadder I	200 Lanes
RiboLadder Short	25 Lanes
RiboLadder Long	25 Lanes

One-Step RT-PCR 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
- It is necessary to use a suitable RNA extraction reagent

Primer Design and Concentration

- The use of gene-specific primers is recommended for use with the One-Step RT-PCR Kit. The use of oligo dT or random hexamers is not recommended with a One-Step RT-PCR set-up since this can result in the generation of non-specific products
- In most cases a final primer concentration of 200nM is sufficient. However, we recommend a primer titration within the 50-500nM range
- Primers should be checked to ensure that they are not self-complementary
- Primer design can benefit from the use of an RNA secondary structure prediction model (e.g. MFOLD), to ensure that priming is not prevented by internal double-stranded regions caused by folding
- The use of intron-spanning primers allows differentiation between amplified cDNA and contaminating genomic DNA
- Annealing temperature of primers is usually melting temperature (T_m) minus 5-10°C

MgCl₂ Optimization

- The final reaction will contain 1.5mM MgCl₂ (the 2x One-Step RT-PCR buffer contains 3mM MgCl₂), which should be optimal for most reverse transcriptase and PCR reactions
- MgCl₂ requirements for the reaction can vary, depending on the particular template and primers used
- A titration of MgCl₂ can be performed to optimise the reaction conditions
- The table below shows how much of the 50mM MgCl₂ solution (provided) should be added to each reaction to provide an elevated concentration in the final reaction

Volume of 50mM MgCl ₂ to be added to a 50µl reaction	Final concentration of MgCl ₂
0	1.5mM
0.5µl	2.0mM
1.0µl	2.5mM
1.5µl	3.0mM
2.0µl	3.5mM
2.5µl	4.0mM
3.0µl	4.5mM
3.5µl	5.0mM
4.0µl	5.5mM

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
- Heat-activated enzyme require an activation period of 10 minutes at 95°C is required prior to the cycling steps.

- 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.

One-Step RT-PCR Protocol

1) Assemble the following components on ice in a certified RNase-free reaction tube:

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		

2) Program the Thermal Cycler to include the RT and subsequent PCR step:

3)

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

4) Mix reactions gently, load into thermal cycler and start reaction.

5) Analyse the amplified product.

RT-PCR 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).
Target not expressed in tissue analyzed:		Try a different target of tissue.
Poor Specificity	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.
Genomic DNA contamination:		Try a different target of tissue.

Suggested Controls

Performing the following controls may prove useful in validating your results.

1. Control for DNA contamination (no RT control): Set up a standard 1-step reaction and start at the 10 min 95°C step (to denature RT and activate DNA Polymerase) followed by normal PCR cycling for 40 cycles:

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	Extensio

The presence of a product suggests DNA contamination and the sample should be DNase I treated.

2. Control for cDNA synthesis: Remove the sample from the thermocycler after the 15-30 minutes at 37°C to 55°C step, and analyze by agarose gel electrophoresis to confirm synthesis.

3. Positive control for PCR amplification: Use a genomic template DNA (not provided) to set up a standard reaction starting at 10 min 95°C followed by normal PCR cycling for 40 cycles as in table above.