

# RobusT RT-PCR

The new  
**RobusT RT-PCR Kit**

from Finnzymes is a  
High Fidelity One-Tube  
RT-PCR system for convenient,  
sensitive, and reproducible  
RT-PCR amplification of  
a specific target RNA

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PRODUCTS FOR PCR AND MOLECULAR BIOLOGY

# HIGH FIDELITY ONE-

*High Fidelity One-Tube RT-PCR system for convenient, sensitive, and reproducible RT-PCR\* amplification of a specific target RNA*

## KIT DESCRIPTION

The RobusT RT-PCR Kit is designed for convenient, sensitive and reproducible amplification of a specific target RNA up to 6.5 kb in length. The excellent performance of the system is based on AMV Reverse Transcriptase (AMV-RT) in the first strand cDNA synthesis and on DyNAzyme EXT DNA polymerase in the second strand synthesis and following DNA amplification. Complementary DNA synthesis and high-fidelity PCR amplification of cDNA are performed successively in a single tube during a continuous thermal-cycling program.

Compared to other RT-PCR protocols (uncoupled RT-PCR, discontinuous one-tube RT-PCR), continuous RT-PCR is the most efficient system. The risk of contamination is minimized by combining cDNA synthesis and PCR, and eliminating component addition following cDNA synthesis (1). The one-tube one-buffer system minimizes hands-on time required. Both total RNA and mRNA can be used as template for the first strand synthesis. One-step RT-PCR format is useful for running multiple reactions simultaneously for high-throughput screening of RNA samples.

## APPLICATIONS OF RobusT RT-PCR SYSTEM

- Robust, high yield RNA amplification
- Reliable expression profiling
- Generation of cDNA products with high fidelity for cloning and sequencing
- Quantification of gene expression

\*) The PCR process is covered by patents owned by Hoffmann-La Roche

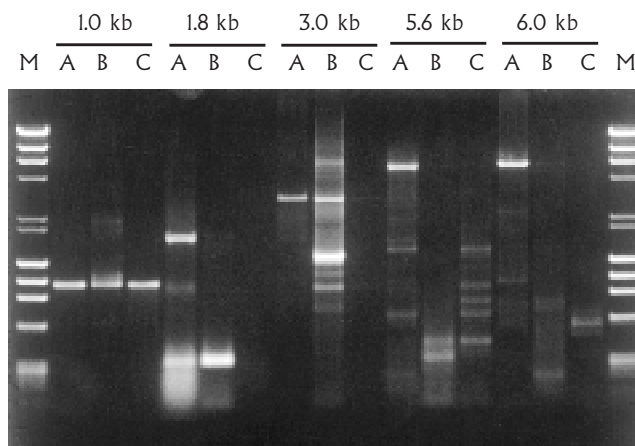
## ADVANTAGES OF RobusT RT-PCR SYSTEM

- Robust system with high fidelity due to optimal AMV RT / DyNAzyme EXT combination; see example 1
- Capable of amplifying long targets, up to 6.5 kb; see example 2
- High sensitivity; see example 3
- One-tube system allows quick, sensitive and reproducible analysis of RNA with minimal risk of sample contamination
- Minimal hands-on time required
- Amplifies products from a wide variety of total RNA or mRNA sources; see example 1
- Saves time by eliminating the need to construct and screen cDNA libraries

### Example 1.

#### Robust and reliable system with high fidelity

The RobusT RT-PCR Kit produced stronger bands than competing enzyme combinations, with a variety of RNA targets and lengths.



*Figure 1. Comparison of various RT-PCR enzyme combinations with RobusT RT-PCR system*

RT-PCR systems used in the comparison above:

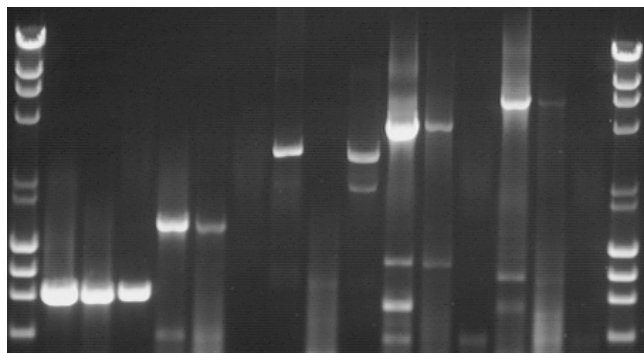
- A. RobusT AMV-RT + DyNAzyme EXT
- B. Competitor B. MMLV-RT and *Taq*-based enzyme mix
- C. Competitor C. RNase H<sup>-</sup> MMLV-RT and *Taq*-based enzyme mix

RNA targets used in the comparison presented in figure 1.

Lanes	Target	Source
M	$\lambda$ DNA / <i>Hind III</i> + $\phi$ X174 / <i>Hae III</i> Digest	
1-3	1.0 kb MS2 bacteriophage	10 ng RNA
4-6	1.8 kb dystrophin	(1 $\mu$ g human skeletal muscle total RNA)
7-9	3.0 kb dystrophin	(1 $\mu$ g human skeletal muscle total RNA)
10-12	5.6 kb dystrophin	(1 $\mu$ g human skeletal muscle total RNA)
13-15	6.0 kb dystrophin	(1 $\mu$ g human skeletal muscle total RNA)
M	$\lambda$ DNA / <i>Hind III</i> + $\phi$ X174 / <i>Hae III</i> Digest	

# TUBE RT-PCR SYSTEM

0.8 kb    1.5 kb    3.0 kb    4.1 kb    6.0 kb  
M    A B C    A B C    A B C    A B C    A B C    M



**Figure 2. Amplification of RNA targets of different origin with RobusT RT-PCR system and competing one-tube, one buffer systems**

RT-PCR systems used in the comparison above:

- A. RobusT RT-PCR system
- B. Competitor D
- C. Competitor E

Note: All enzymes were used in optimal conditions provided in the systems and according to instructions included in the product manuals.

RNA targets used in the comparison presented in figure 2.

Lanes:

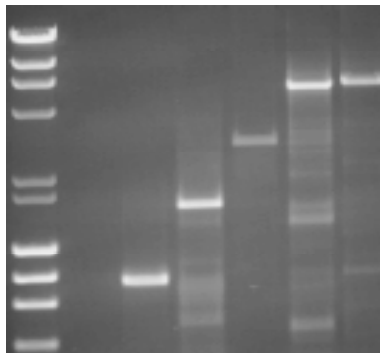
- M    λ DNA / *Hind III* + φX174 / *Hae III* Digest
- 1-3    0.8 kb    proteinase K [100 ng *Tritirachium album* total RNA]
- 4-6    1.5 kb    TdT [1 μg calf thymus total RNA]
- 7-9    3.0 kb    10 ng MS2 RNA
- 10-12    4.1 kb    dystrophin [1 μg human skeletal muscle total RNA]
- 13-15    6.0 kb    dystrophin [1 μg human skeletal muscle total RNA]
- M    λ DNA / *Hind III* + φX174 / *Hae III* Digest

In all comparisons presented in figures 1 and 2, the RobusT RT-PCR system provided the best and most consistent results. With RobusT RT-PCR system strong and reliable product bands are obtained under a variety of conditions.

## Example 2. Broader working range with the RobusT RT-PCR system

The RobusT RT-PCR system is ideal for long transcripts and a wide range of reaction conditions. In Figure 3, the RobusT RT-PCR system yielded reliable product bands over a broad range of templates from 1.0 kb up to 6.0 kb in length. The RobusT RT-PCR system amplifies RNA templates from a wide variety of total RNA or poly(A)<sup>+</sup> RNA sources.

M    1.0 kb 1.8kb 3.0kb 5.6kb 6.0kb



**Figure 3. Broad range of target RNA lengths amplified with RobusT RT-PCR system**

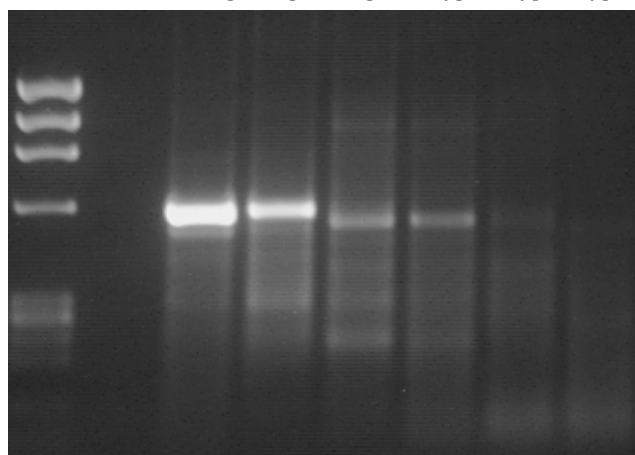
Lanes applicable to figure 3:

- M    λ DNA / *Hind III* + φX174 / *Hae III* Digest
- 1    1.0 kb    MS2 bacteriophage 10 ng RNA
- 2    1.8 kb    dystrophin [1 μg human skeletal muscle total RNA]
- 3    3.0 kb    dystrophin [1 μg human skeletal muscle total RNA]
- 4    5.6 kb    dystrophin [1 μg human skeletal muscle total RNA]
- 5    6.0 kb    dystrophin [1 μg human skeletal muscle total RNA]

## Example 3. Higher sensitivity for low abundance RNA

**Figure 4. Detection of a low abundance target from total RNA with RobusT RT-PCR system (Human ADP Ribosylation Factor 1)**

M    100ng 10ng 1ng 100pg 10pg 1pg



Lanes applicable to figure 4:

- M    λ DNA / *Hind III* + φX174 / *Hae III* Digest
- 1    100 ng of human skeletal muscle total RNA
- 2    10 ng of human skeletal muscle total RNA
- 3    1 ng of human skeletal muscle total RNA
- 4    100 pg of human skeletal muscle total RNA
- 5    10 pg of human skeletal muscle total RNA
- 6    1 pg of human skeletal muscle total RNA

## The RobusT RT-PCR system is easy to optimize and easy to use

First strand cDNA synthesis overcomes secondary structure and nonspecific priming problems

Using AMV reverse transcriptase, the reverse transcription step can be carried out even at elevated temperatures up to 60 °C. High temperature increases the specificity of priming and increases the efficiency of the reaction by reducing RNA secondary structures.

Unique advantages of DyNAzyme EXT provide unsurpassed fidelity and robustness

Thermostable DNA polymerases lacking proofreading activity are incapable of removing misincorporated nucleotides introduced by the polymerase and thus in most cases are not able to continue the polymerization reaction. This phenomenon reduces the amount of final product formed. That is why a low amount of proofreading activity is needed to remove the misincorporations which could terminate DNA synthesis.

In the RobusT RT-PCR system the second strand cDNA synthesis and following high fidelity PCR amplification is performed by DyNAzyme EXT DNA Polymerase, which is a versatile and easy-to-use enzyme with powerful advantages over *Taq* polymerase for all PCR applications. DyNAzyme EXT is an optimal mixture of recombinant DyNAzyme II DNA polymerase (*Thermus brockianus*) and a proofreading enzyme. DyNAzyme EXT contains sufficient proofreading activity to remove misincorporated nucleotides, yielding good efficiency as well as high fidelity. DyNAzyme EXT exhibits fidelity equal to the best of traditional cloning enzymes but with a much broader working range and ease of optimization.

(See [www.finnzymes.fi](http://www.finnzymes.fi) for more information)

## STORAGE STABILITY

Store all kit components at -20 °C. For long term storage, the control RNA and AMV reverse transcriptase may be stored at -70 °C. The RobusT RT-PCR system is stable for one year from the date of packaging when stored and handled properly.

## RobusT RT-PCR KIT COMPONENTS

The kit contains sufficient materials for 100 (F-580L) or 20 reactions (F-580S) and for 20 control reactions.

COMPONENT	CONCENTRATION
10 X RobusT Reaction Buffer	
MgCl <sub>2</sub> solution	50 mM
dNTP mix	10 mM each
AMV Reverse Transcriptase	5 U/μl
DyNAzyme EXT DNA Polymerase	1 U/μl
Upstream Control primer	10 pmol/μl
Downstream Control primer	10 pmol/μl
Control RNA	20 μl

## ORDERING INFORMATION

### RobusT RT-PCR Kit

F-580S	20 reactions/20 control reactions
F-580L	100 reactions/20 control reactions

Individual components also separately available

### AMV Reverse Transcriptase

F-570S	500 U	20 U/μl
F-570L	2500 U	20 U/μl

DyNAzyme EXT DNA Polymerase with Optimized EXT Buffer and Buffer Pack for Mg<sup>+</sup> Optimization

F-505S	200 U
F-505L	1000 U

### PCR License Disclaimer

These products are sold under licensing arrangements of Finnzymes Oy with F. Hoffmann-La Roche Ltd, Roche Molecular Systems, Inc. and The Perkin-Elmer Corporation. The purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler.

## PRODUCTS FOR PCR AND MOLECULAR BIOLOGY



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