RobusT RT-PCR

One-tube Systems

RobusT I Kit

High thermostability with AMV RT

RobusT II Kit

Excellent sensitivity with M-MuLV RNase H⁻ RT

FINNZYMES

PRODUCTS FOR PCR AND MOLECULAR BIOLOGY

Single-tube systems for convenient, sensitive and reproducible RT-PCR* amplification of a specific target RNA

RT-PCR, coupling reverse transcription (RT) of mRNA with PCR amplification of the resulting cDNA, is a rapid and sensitive method for analyzing gene expression and producing cDNA for cloning.

RobusT RT-PCR Kits are designed to improve the sensitivity and efficiency of cDNA synthesis and PCR amplification. The reactions are designed for a convenient one-tube- one-buffer system. A unique reverse transcriptase/DNA polymerase combination in two different kit formats guarantees the excellent features of the kits. The RobusT I kit is based on the highly thermostable AMV RT, capable of producing cDNA at elevated temperatures and thus minimizing the problems originating from secondary RNA structures. The RobusT II kit is based on the sensitive properties of M-MuLV RNase H$^-$ (minus) facilitating the amplification of minor amounts of starting mRNA material. The excellent performance of the systems is then built into DyNAzyme™ EXT DNA polymerase, which has proved to be a powerful amplification enzyme capable of high fidelity DNA synthesis with high yields.

One-Tube RobusT RT-PCR protocol

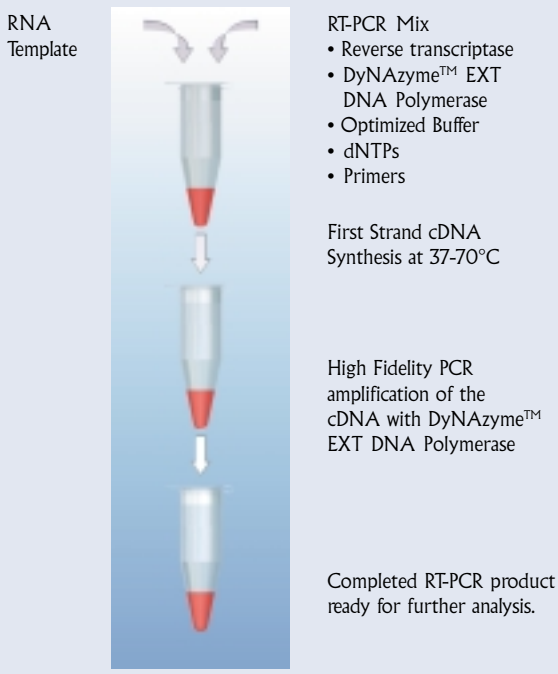

Figure 1.

RobusT RT-PCR Kits perform cDNA synthesis and PCR amplification of cDNA successively in a single tube during a continuous thermal cycling. Use of the same buffer conditions and site-specific primers eliminates the need to open the reaction tube and adjust conditions between the RT and PCR steps. In addition to increased sensitivity, the one-tube system minimizes the risk of contamination and the requirement for hands-on time.

APPLICATIONS OF RobusT RT-PCR SYSTEMS

- Robust, high-yield RNA amplification
- Generation of cDNA products with high fidelity for cloning, sequencing and microarray purposes
- Quantification of gene expression and reliable expression profiling
- Clinical diagnostics: detection and quantification of infectious microorganisms, cancer cells and genetic disorders

ADVANTAGES OF RobusT RT-PCR SYSTEMS

- One-tube system allows rapid, sensitive and reproducible analysis of RNA with minimal risk of sample contamination (Figure 1)
- Capable of amplifying long targets, up to 6.0 kb (Figure 2)
- Amplifies products from a wide variety of total RNA or mRNA sources (Figure 2)

Broad range of target RNAs amplified with RobusT RT-PCR systems

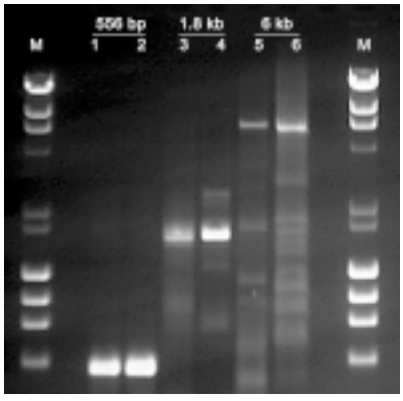

Figure 2.

RT-PCR systems used in the comparison (figure 2)
Lanes 1, 3, 5: RobusT I, AMV RT + DyNAzyme™ EXT DNA Polymerase
Lanes 2, 4, 6: RobusT II, M-MuLV RNase H$^-$ RT + DyNAzyme™ EXT DNA Polymerase

Marker: λ DNA/*Hin*d III + ϕX174/*Hae* III Digest
RNA: human skeletal muscle total RNA
Lanes 1, 2: 556 bp Human ADP Ribosylation Factor 1
Lanes 3, 4: 1,8 kb dystrophin
Lanes 5, 6: 6 kb dystrophin

RobusT I RT-PCR KIT

AMV RT / DyNAzyme EXT

A CHOICE FOR PROBLEMATIC RNA

RobusT I Kit is a combination of Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT) and DyNAzyme™ EXT DNA Polymerase.

RobusT I RT-PCR Kit offers the best combination of enzymes for amplifying templates with difficult secondary structures. Due to the increased thermostability of AMV RT the cDNA synthesis step can be performed at elevated temperatures up to 70°C, which helps to overcome secondary structure problems. In addition the use of elevated temperatures improves the specificity of reverse transcription by decreasing false priming.

High thermostability of AMV RT

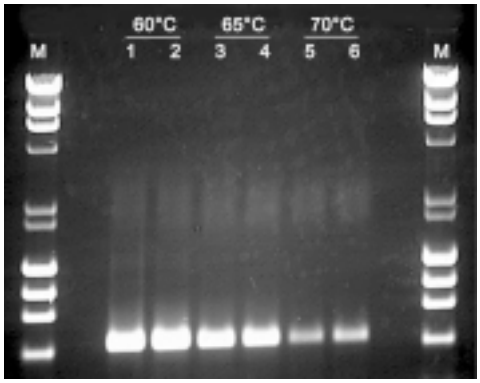

Figure 3.

RNA: Human skeletal muscle total RNA
Target: Human cytoskeletal gamma actin, 625 bp
Marker: λ DNA / *Hin*d III + ϕX174 / *Hae* III Digest
cDNA synthesis temperature 60°C - 70°C

COMPONENTS of RobusT I RT-PCR KIT

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

COMPONENT	CONCENTRATION
10 X RobusT Reaction Buffer	
$MgCl_2$ 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 with Carrier	10 ng MS2 RNA/µl 30 µg *E. coli* ribosomal RNA/ml

RobusT II RT-PCR KIT

M-MuLV RNase H⁻ RT / DyNAzyme EXT

A CHOICE FOR LOW ABUNDANCE RNA

RobusT II Kit is a combination of Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase, RNase H⁻ (minus) and DyNAzyme™ EXT DNA Polymerase for higher sensitivity.

M-MuLV H⁻ (minus) RT is purified from a recombinant source and due to the absence of RNase H activity it yields much larger quantities of full-length cDNA transcripts than wild type M-MuLV RT, which possesses substantial RNase H activity. RobusT II RT-PCR Kit offers an optimal enzyme combination for synthesis of long cDNAs from a variety of RNA sources and target sizes.

High sensitivity for low abundance RNA

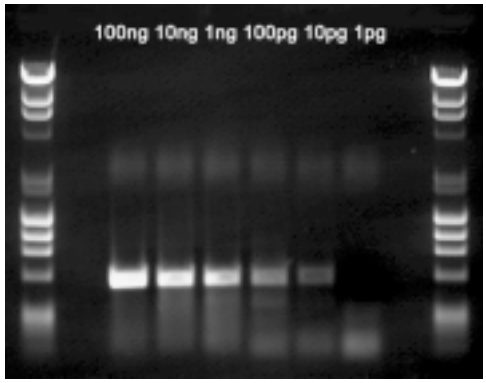

Figure 4.

RNA: Human skeletal muscle total RNA
Target: Human ADP Ribosylation Factor 1 (556 bp) (low copy gene)
Marker: λ DNA / *Hin*d III + ϕX174 / *Hae* III Digest
Each successive lane represents decreasing amounts of total RNA from human skeletal muscle ranging from 100 nanograms to 1 picogram.

COMPONENTS of RobusT II RT-PCR KIT

The kit contains sufficient materials for 100 (F-590L) or 20 reactions (F-590S).

COMPONENT	CONCENTRATION
10 X RobusT Reaction Buffer	
$MgCl_2$ solution	50 mM
dNTP mix	10 mM each
M-MuLV Reverse Transcriptase, RNase H⁻ (minus)	5 U/µl
DyNAzyme™ EXT DNA Polymerase	1 U/µl
Upstream Control primer	10 pmol/µl
Downstream Control primer	10 pmol/µl
Control RNA with Carrier	10 ng MS2 RNA/µl 30 µg *E. coli* ribosomal RNA/ml

INDIVIDUAL ENZYMES AVAILABLE SEPARATELY

M-MuLV RNase H- Reverse Transcriptase

Moloney Murine Leukemia Virus (M-MuLV) RNase H- (minus) Reverse Transcriptase is an RNA-directed DNA polymerase. It is purified from a strain of *E.coli* carrying the reverse transcriptase gene from M-MuLV, modified by site directed mutagenesis to eliminate the RNase H activity. RNase H activity is presumed to be responsible for decreasing the length and yield of cDNA products. M-MuLV RNase H- RT has improved efficiency for full length cDNA synthesis.

M-MuLV RNase H- RT is supplied with optimized 10 x M-MuLV Buffer.

AMV Reverse Transcriptase

The AMV Reverse Transcriptase (AMV RT) is isolated from Avian Myeloblastosis Virus. Using AMV Reverse Transcriptase, the reverse transcription step can be carried out at elevated temperatures up to 70°C. High temperature increases the specificity of priming and helps to increase the efficiency by reducing RNA secondary structures.

AMV RT is supplied with optimized 10 x AMV Buffer.

DyNAzyme™ EXT DNA Polymerase

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

DyNAzyme™ EXT DNA Polymerase is supplied with optimized 10 x EXT Buffer.

STORAGE STABILITY

Store all kit components at -20°C. For long term storage, the control RNA and AMV Reverse Transcriptase should be stored at -70 °C. The RobusT RT-PCR systems are stable for one year from the date of packaging when stored and handled properly.

ORDERING INFORMATION

RobusT I RT-PCR Kit
F-580S 20 reactions
F-580L 100 reactions

RobusT II RT-PCR Kit
F-590S 20 reactions
F-590L 100 reactions

AMV Reverse Transcriptase
With Optimized 10 x AMV Buffer
F-570S 500 U 20 U/µl
F-570L 2,500 U 20 U/µl

M-MuLV RNase H- Reverse Transcriptase
With Optimized 10 x M-MuLV Buffer
F-572S 10,000 U 200 U/µl
F-572L 50,000 U 200 U/µl

DyNAzyme ™ EXT DNA Polymerase
With Optimized EXT Buffer and Buffer Pack for Mg 2+ Optimization
F-505S 200 U 1 U/µl
F-505L 1,000 U 1 U/µl

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