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Description

RT-PCR is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step Taq DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. During subsequent rounds of cycling the DNA polymerase exponentially amplifies this double-stranded DNA template.

LYO RT-PCR Mastermix is designed for performing maximum sensitive and specific RT-PCRs convenient in single tubes. The enzyme mix is based on a genetically engineered RT polymerase with enhanced thermal stability resulting in an increased specificity, higher cDNA yield and an improved efficiency for highly structured and long cDNA fragments.

The lyophilized Mastermix contains all reagents required for successful RT-PCR (except template and primer) in one tube to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzyme mix, ultrapure dNTPs and the optimized complete reaction buffer ensure superior amplification results in simple steps.

In LYO RT-PCR Mastermix all components of RT and PCR are mixed in one tube prior to starting the reaction and thus carried out sequentially without opening the tube. This offers tremendous convenience when applied to analysis of single targets from multiple samples of RNA and minimizes the risk of contaminations.

Handling

LYO RT-PCR Mastermix is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete qPCR master mix in a dry, room temperature stable format.
The lyophilized Mixture combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.
Each vial contains all components (except primers and template) required for a 20 µl RT-PCR assay. To perform the assay, only fill up the vials with a mix of primers and RNA template.

Storage and shipping temperature: between 5 – 25 °C, minimum shelf life: 16 months

Manufactured and quality-controlled in accordance with ISO 9001:2000

LYO RT-PCR Mastermix contains:

Preloaded lyophilizates containing Reverse Transcriptase, Hot Start Polymerase, dNTPs, Reaction Buffer, MgCl2 and stabilizers, **PCR-grade water**

Sensitivity

Targets can generally be detected from 10 pg to 500 ng polyA RNA or 10 pg to 1µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

Preparation of the RNA/Primer Mix

Mix all components gently by pipetting up and down. To perform the assay, only fill up the vials with a mix of primers and RNA template.

Component	stock-concentration	final concentration	1 assay
RNA Template		10 pg- 1 µg	
Forward primer	10 µM	400-600 nM	0,8-1,2 µl
Reverse primer	10 µM	400-600 nM	0,8-1,2 µl
RNase-free water			up to 20 µl

2. Denaturation and primer annealing (optional)

For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results. Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure and for self- or cross-complementary primers and for initial experiments with new targets. Incubate the mixture at 70°C for 5 min and place it at room temperature for 5 min.

3. Dispensing the master mix

Dispense 20 µl of the RNA/Primer Mix to each PCR tube or well of the plate on request.

Reverse transcription and thermal cycling

Place the vials in a PCR cycler and start the following program

Reverse transcription 1)	50 °C	30-60 min	1x
Initial denaturation 2)	95 °C	5 min	1x
Denaturation	95°C	10-20 sec	30-40x
Annealing 3)	55-65°C	20-30 sec	30-40x
Elongation 4)	72°C	1 min/kb	30-40x
Final elongation	72°C	5 min	1x

Notes:

1) The optimal time depends on the length of cDNA. Incubation of 60 min is recommended for cDNA fragments of more than 2,000 bp length. The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.

2) A prolonged initial denaturation time of up to 5 min is recommended to inactivate the reverse transcriptase

3) The annealing temperature depends on the melting temperature of the primers.

4) The elongation time depends on the length of the amplicon. A time of 1 min for a fragment of 1,000 bp is recom-

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Order Information

Prod. No.	Description	Quantity
1905-600	Lyophilized Mastermix for RT-PCR	12*8 Tubes stripes
1905-605	Lyophilized Mastermix for RT-PCR	60*8 Tubes stripes