



One-Step RT-PCR Master Lyophilisate

lyophilised One-Step RT-PCR Master Mix

Cat. No.	Amount
PCR-159S	192 reactions x 20 µl
PCR-159L	960 reactions x 20 µl

For *in vitro* use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Additional Storage Conditions: Store in an aluminium-coated bag or on a dry place.

Lyophilisates may hydrate at humidity levels >70 % when sealing is opened.

Shelf Life: 12 months

Description:

One-Step RT-PCR Master Lyophilisate 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 lyophilisate contains all reagents required for RTPCR (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.

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.

In one-step RT-PCR 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.

Content:

RT Master Lyophilisate

Preloaded lyophilisates containing SCRIPT Reverse Transcriptase, Hot Start Polymerase Ab+, dNTPs, Reaction Buffer, MgCl₂ and stabilizers.

PCR grade water

Handling

One-Step RT-PCR Master Lyophilisate 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 lyophilisate 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.

Sensitivity

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



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RT-PCR assay preparation

1. Preparation of the RNA/Primer Mix

Add the following components to a nuclease-free microtube and mix by pipetting gently up and down:

Component	stock conc.	final conc.	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			fill up to 20 µl

2. Denaturation and primer annealing (optional)

Please note: Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure, for self- or cross-complementary primers and for initial experiments with new targets. For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results.

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 lyophilisate containing tube or well of the plate.

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	
Elongation ⁴⁾	72 °C	1 min/kb	
Final Elongation	72 °C	5 min	1x

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 1000 bp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary.