Data Sheet

IFTA AG
Certified QMS according to
DIN EN ISO 9001
Reg. No. IC 03214 034

Jena Bioscience

One-Step RT-PCR Master Lyophilisate

Lyophilized master mix for One-Step RT-PCR

Ready-to-use lyophilisates

	Cat. No.	Amount	Size
	PCR-159S-8TS	12 strips / 96 reactions	8-tube strips
	PCR-159L-8TS	60 strips / 480 reactions	
	PCR-159S-FTP	2 plates / 192 reactions	96-well plates (flat top, without skirt)
	PCR-159L-FTP	10 plates / 960 reactions	
	PCR-159S-HSP	2 plates / 192 reactions	96-well plates (half skirt)
	PCR-159L-HSP	10 plates / 960 reactions	

For *in vitro* use only
Quality guaranteed for 12 months
Store below 25°C
Store in an aluminium-coated bag or on a dry place
Lyophilisates may hydrate at humidity levels >70% when sealing is opened

One-Step RT-PCR Master Lyophilisate
Preloaded lyophilisates containing SCRIPT Reverse Transcriptase, Hot Start Polymerase, dNTPs, Reaction Buffer, $MgCl_2$ and stabilizers

PCR-grade water

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 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.
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.

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.

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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.

RT-PCR assay preparation

1. Preparation of the RNA/Primer Mix
Add the following components to a nuclease-free microtube and mix the components 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 PCR 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	30-40 x
annealing [3]	55-65°C	20-30 sec	
elongation [4]	72°C	1min/kb	
final elongation	72°C	5min	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 1,000 bp is recommended.

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