DATA SHEET

cellco
Biotecnologia ao seu alcance

RT-PCR Master (2x)

One-Step RT-PCR Master Mix (2x conc.) for highly sensitive and specific amplification.

Cat. Nº.	Amount
☐ PRT-120XS	1, 25 ml
☐ PRT-120S	2 x 1,25 ml
☐ PRT-120M	4 x 1,25 ml
☐ PRT-120L	10 x 1,25 ml

Concentration:
Master Mix 2x concentrated

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C (Avoid freeze/thaw cycles, store in dark).

For in vitro use only!

Form:
Liquid

Shelf Life:
12 months

Description:
RT-PCR Master Mix is designed for performing highly sensitive and specific RT-PCR convenient in single tubes. The enzyme mix is based on a genetically engineered reverse transcriptase with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments. The 2 x conc. mix 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. Premium quality enzymes in combination with ultrapure dNTPs and an advanced buffer system 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 hot start 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 so that the complete reaction can be performed without opening the tube. This offers tremendous convenience when applied in routine testing and minimizes the risk of contaminations. RT-PCR Master contains RNase inhibitor that is essential when working with low amounts of starting RNA.

Sensitivity:
Targets can generally be detected from <1 pg to 20 ng poly(A) RNA (mRNA) or 10 pg to 1 µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

Content:

RT-PCR Master Mix
2x conc. master mix containing reverse transcriptase, hot start polymerase chemically modified, RNase inhibitor, dNTPs, reaction buffer, additives and stabilizers.

PCR-grade Water

Preparation of the RT-PCR Assay

RT-PCR assay without sample denaturation
Recommended for most standard combinations of template RNA and primers, sample denaturation can be omitted with no negative effect on results.

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

DATA SHEET

cellco
Biotecnologia ao seu alcance

RT-PCR Master (2x)

One-Step RT-PCR Master Mix (2x conc.) for highly sensitive and specific amplification.

Component	20 µL assay	Final [ ]
RT-PCR Master Mix 2x	10 µL	1x
Primer forward (10 µM)	1-2 µL	200-400 nM
Primer reverse (10 µM)	1-2 µL	200-400 nM
RNA Template[1]	x µL	1 pg - 1 µg
RNAse free water	to 20 µL	-

1) 1 ng to 500 ng polyA RNA or 10 ng to 5 µg total RNA

Continue with reverse transcription and thermal cycling as recommended.

RT-PCR assay with sample denaturation

Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure, for self- or crosscomplementary primers and for initial experiments with new targets

Preparation of the RNA Template / Primer Mix

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

Component	20 µL assay	Final [ ]
Primer forward (10 µM)	1-2 µL	200-400 nM
Primer reverse (10 µM)	1-2 µL	200-400 nM
RNA Template[1]	x µL	1 pg - 1 µg
RNAse free water	to 20 µL	-

Denaturation and primer annealing

Incubate the mixture at 70 °C for 5 min and place it at room temperature for 5 min.

Preparation of the RT-PCR Mix

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

Component	20 µL assay	Final [ ]
RNA Template/primer Mix	10 µL	-
RT-PCR Master Mix 2x	10 µl	1x

Reverse transcription and thermal cycling:

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

Step	Temp.	Time	Cycle
Reverse Transcription[2]	55 °C	10 min	1x
Initial denaturation[3]	95 °C	5 min	1x
Denaturation	95 °C	10 sec	
Annealing[4]	55-65 °C	20 sec	30 -40x
Elongation[5]	72 °C	1 min-kb	
Final elongation	72 °C	5 min	1x

2) The optimal time depends on the length of cDNA. Incubation of 10 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 65 °C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular

RNA.

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

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

5) 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. Note that optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.