Datasheet

One.Step RT-PCR Master Mix (2X) with SybrGreen (no-ROX / high-ROX)

Cat. without ROX: 105-520, 105-520L, high ROX: 105-530, 105-530L

For quantitative real-time analyses of RNA templates using SybrGreen as fluorescent dye

Description

The One.Step RT-PCR Master Mix is designed for quantitative real-time analyses of RNA templates using SybrGreen as Fluorescent Stain. The enzyme mix is based on a with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments.

The 2X conc. mix contains all reagents required for RT-qPCR (except template and primers) to ensure fast and easy preparation with a minimum of pipetting steps.

RT-qPCR is used to amplify double-stranded DNA from single-stranded RNA templates to allow a rapid real-time quantification of RNA targets. In the reverse transcription step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template.

In the first cycle of the PCR step the hot-start DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. The hot-start polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.

One.Step RT-PCR Master Mix offers tremendous convenience when applied to analysis of targets from multiple samples of RNA and minimizes the risk of contaminations.

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.

SybrGreen® Fluorescent DNA Stain

SybrGreen® Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing only the lowest inhibition to the PCR process. The dye is stable both thermally and hydrolytically, providing convenience during routine handling.

Spectroscopic data SybrGreen®: Excitation maximum: λ_{Exc} = 494 nm (bound to DNA); Emission maximum: λ_{Em} = 521 nm (bound to DNA). Just select the optical settings for SybrGreen on the cycler platform.

ROX reference dye included only in Cat. No. 105-530, 105-530L

One.Step RT-PCR Master Mix (2X) high ROX contains 500 nM Rox passive reference dye in the final assay. The ROX does not take part in the PCR reaction but allows to normalize for non-PCR related signal variation and provides a baseline in multiplex reactions.

Components

One.Step RT-PCR Master Mix (2X):

The Mastermix contains: Reverse Transcriptase, Hot Start Polymerase, RNase Inhibitor, dNTPs, reaction buffer, SybrGreen fluorescent DNA stain and stabilizers, optional no ROX / high ROX.

RNase-free water: included

RT-PCR assay without sample denaturation (standard RNA/primer combinations)

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

.. a good decision ..

component	stock conc.	final conc.	20 µl assay	50 µl assay
RNase-free water			fill up to 20 µl	fill up to 50 µl
RNA Template[1)]		< 100 ng	X µl	X µl
forward Primer	10 µM	400 nM	0.8 µl	2 µl
reverse Primer	10 µM	400 nM	0.8 µl	2 µl
One.Step RT-PCR Master Mix[2)]	2x	1x	10 µl	25 µl

[1)] up to 100 ng polyA RNA or total RNA
[2)] One.Step RT-PCR Master Mix already contains RNase inhibitor that may be essential when working with low amounts of starting RNA. Continue with reverse transcription and thermal cycling as recommended.

Reverse transcription and thermal cycling Place the vials in a PCR cycler and start the following program.

Reverse transcription [3)]	50-55°C	10-15 min	1x
Initial denaturation [4)]	95°C	5 min	1x
Denaturation	95°C	15 sec	35-45 x
Annealing [5)]	55-65°C	20 sec	35-45 x
Elongation [6)]	72°C	30 sec	35-45 x

[3)] A reverse transcription time of 10 min is recommended for optimal amplicon lengths between 100 and 200 bp. Longer amplicons up to 500 bp may require a prolonged incubation of 15 min. Add 3 min for each additional 100 bp. 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.
[4)] An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase
[5)] The annealing temperature depends on the melting temperature of the primers.
[6)] The elongation time depends on the length of the amplicon. A time of 1 min for amplicons up to 1,000 bp is recommended.

Storage:
@ -20°C, avoid frequent thawing and freezing, store all components in the dark; stable @ 4°C for up to 4 weeks

Transport:
the product will be shipped with "blue ice"

Ordering information

Cat.-no	Description	Amount
105-520	One.Step RT-PCR Master Mix (2x) SybrGreen no ROX	2 x 1,25 ml
105-520L	One.Step RT-PCR Master Mix (2x) SybrGreen no ROX	10 x 1,25 ml
105-530	One.Step RT-PCR Master Mix (2x) SybrGreen high ROX	2 x 1,25 ml
105-530L	One.Step RT-PCR Master Mix (2x) SybrGreen high ROX	10 x 1,25 ml

As an alternative to avoid cross contamination, please refer to One.Step RT-PCR Mastermix with dUTP/UNG. Product code: 105-526, 105-528.

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.

.. a good decision ..