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Features:

- The kit contains all reagents required for RT-qPCR in a set to ensure fast and easy preparation with a minimum of pipetting steps. Just add template, primers. That's all.
- The One.Step RT-PCR Kit is highly sensitive: less than 10 pg to 100 ng total RNA or < 1 pg to 20 ng poly(A) RNA (mRNA) can be detected when using highly expressed transcripts

Description:

The enzyme mix is based on a new formulated reverse transcriptase, a Hot-Start Polymerase and RNase Inhibitor. The set is providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments. The reaction buffer includes extrapure dNTPs, EvaGreen as intercalating dye and reaction enhancers.

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.

EvaGreen® Fluorescent DNA Stain

EvaGreen® Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR) and high-resolution DNA melting curve analysis (HRM). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing no detectable inhibition to the PCR process. The dye is extremely stable both thermally and hydrolytically, providing convenience during routine handling.

Spectroscopic data EvaGreen®: Excitation maximum: λExc = 500 nm (bound to DNA); Emission maximum: λEm = 530 nm (bound to DNA). Just select the optical settings for Sybr Green on the cycler platform.

Platforms: The Kit is suitable for all block-based Thermocycler. Stringent Quality Tests on ABI StepOne plus PCR Cycler

Components:

Maximo.OneStep RT-qPCR Kit Mastermix (red cap): 2x concentrated Mastermix 2 x 1,25 ml or 10 x 1,25ml for L-Pack. (The Mastermix contains: Reverse Transcriptase, Hot Start Polymerase, RNase Inhibitor, dNTPs, reaction buffer, EvaGreen fluorescent DNA stain and stabilizers)

RNase-free water (white cap): 2 x 1,2 ml or 2 x 6 ml for L-Pack

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

1. Preparation of the RT-PCR Assay

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.

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 Template1)		< 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
MAXIMO RT-qPCR Master Mix	2x	1x	10 µl	25

1) up to 100 ng polyA RNA or total RNA

2) MAXIMO RT-qPCR Enzyme 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.

RT-PCR assay with sample denaturation (RNA/primer with a high degree of secondary structure)

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.

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 concentration	final conc.	20 µl assay	50 µl assay
RNase-free water			fill up to 10 µl	fill up to 25 µl
RNA Template1)	< 100 ng	< 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

[1)] up to 100 ng polyA RNA or total RNA

2. Denaturation and primer annealing

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

3. Preparation of the RT-PCR Mix

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

component	stock conc.	final conc.	20 µl assay	50 µl assay
MAXIMO-RT-qPCR -Master	2x	1x	10 µl	25 µl

[2] Maximo.OneStep.-RT-qPCR Enzyme Mix already contains RNase inhibitor that may be essential when working with low amounts of starting RNA.

4. Complete RT-qPCR Mix

Add 15 µl RT-qPCR Mix to 5 µl RNA / Primer Mix to complete the 20 µl assay. Pipette on ice and mix by pipetting gently up and down.

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

Reverse transcription [3]	**50°C**	**10-15 min**	**1x**
Initial denaturation [4]	95°C	5 min	1x
Denaturation	95°C	15 sec	35-45 x
Annealing [5]	60-65°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 with EvaGreen in the dark

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

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Order information:

Prod. No.	Description	Quantity
1905-520	One.Step RT-qPCR with Evagreen	2 x 1,25 ml
1905-522	One.Step RT-qPCR with Evagreen	10 x 1,25 ml