# Datasheet

## MaximoOne.Step RT-qPCR Probes with ROX; DLP-Line

for quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes and ROX

**Description**

The MaximoOne.step RT-qPCR Kit is designed for quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes. 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 kit contains all reagents required for RT-qPCR (except template, primers and the dual labeled fluorescent probe) in one box to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzyme mix and the optimized complete reaction buffer containing ultrapure dNTPs ensure superior real time PCR results.

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-qPCR 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 100 ng total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

**Dual Labeled Fluorescent probes**

Real-time PCR technology based on dual labeled DNA probes provides a highly sensitive and specific PCR system with multiplexing capability. It requires two standard PCR primers and the DNA probe that hybridizes to an internal part of the amplicon. The sequence of the dual labeled DNA probe should avoid secondary structure and primer-dimer formation.
ROX does not take part in the PCR reaction. The dye normalizes the non-PCR signal and gives a baseline.
The kit is optimized for all real-time PCR cyclers who are compatible with the evaluation of the ROX reference signal

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

**Components:**
**Maximo.OneStep RT-qPCR (for probes) Enzyme-mix:** HotStart Taq Polymerase, Reverse Transcriptase, RNase Inhibitor and enhancers, 50 % Glycerol
**Probes Reaction Mix:** Reaction buffer (2X) containing extra pure dNTPs and ROX as reference dye.
**RNase-free water**

**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 | 25 µl assay |
|---|---|---|---|---|
| RNase-free water | | | fill up to 20 µl | fill up to 25 µl |
| RNA Template[1)] | | < 100 ng | X µl | X µl |
| forward Primer | 10 µM | 400 nM | 0.8 µl | 1 µl |
| reverse Primer | 10 µM | 400 nM | 0.8 µl | 1 µl |
| dual-labeled Probe | 10 µM | 200 nM | 0.4 µl | 0.5 µl |
| MAXIMO RT-qPCR Probes-Reaction Mix | 2x | 1x | 10 µl | 12.5 µl |
| RT-qPCR Enzyme Mix [2)] | 25x | 1x | 0.8 µl | 1 µl |

[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 | 25 µl assay |
|---|---|---|---|---|
| RNase-free water | | | fill up to 5 µl | fill up to 5 µl |
| RNA Template1) | | < 100 ng | X µl | X µl |
| forward Primer | 10 µM | 400 nM | 0,8 µl | 1 µl |
| reverse Primer | 10 µM | 400 nM | 0,8 µl | 1 µl |
| dual-labeled Probe | 10 µM | 200 nM | 0,4 µl | 0,5 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 | 25 µl assay |
|---|---|---|---|---|
| RNase-free water | | | fill up to 15 µl | fill up to 20 µl |
| MAXIMO-RT-qPCR Probes-Reaction Mix | 2x | 1x | 10 µl | 12.5 µl |
| MAXIMO RT-qPCR Enzyme Mix [2)] | 25x | 1x | 0.8 µl | 1 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 and elongation | 60-65°C [5)] | 1 min [6)] | 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 and DNA probe used.
[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 ROX in the dark.

**Transport:**

the product will be shipped with "blue ice"

**Ordering information**

| Cat.-no | Description | Amount |
|---|---|---|
| 105-500 | MaximoOne.Step RT-qPCR | 100 rcs / 25µl |
| 105-502 | MaximoOne.Step RT-qPCR | 500 rcs / 25µl |

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.