AMPLIFYME

Probe One-Step No-ROX RT-qPCR Mix

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The ***AMPLIFYME*** Probe One-Step No-ROX RT-qPCR Mix is a convenient reaction mixture for fast and reliable quantitative Real-Time PCR using probes, including TaqMan®, Scorpions® and molecular beacon probes. It was created for reproducible and efficient first-strand cDNA synthesis and subsequent Real-Time PCR in a single tube. It is the best choice for your probe based Real-Time PCR assays, including singleplex and multiplex gene expression studies.

Ready-to-use, 2x concentrated Mix contains all ingredients necessary for Real-Time PCR based on probe detection technology: hot-start Taq polymerase, dNTPs, specially developed buffer, stabilizers and enhancers. Additionally, Mu-MLV Reverse Transcriptase and RNase Inhibitor are included in separate tubes.

The use of high-affinity antibody for hot-start polymerase ensures higher specificity, by reducing formation of primer-dimer structures. It allows to obtain wider dynamic range by removing competition for reaction reagents, it also leads to higher sensitivity and reproducibility.

Precisely optimized buffer components ensures optimal conditions for reverse transcriptase and hot-start polymerase activity. Additionally RNase Inhibitor protects RNA from unspecific RNases.

The ***AMPLIFYME*** Probe One-Step No-ROX RT-qPCR Mix provides fast, highly specific one-step Real-Time RT-PCR results, giving consistent results across all commonly-used Real-Time PCR platforms.

Features and advantages

- → **Versatile** – excellent for various PCR conditions using different Real-Time PCR instruments
- → **Sensitive** – reliable detection of low copies of RNA targets
- → **Reproducible** – consistent amplification across a wide dynamic range
- → **Specific** – precisely selected anti-Taq antibody eliminates non-specific amplification
- → **Efficient** – high efficiency in multiplex reactions
- → **Fast** – accurate detection of molecular targets in as fast as 40 minutes (with either two- or three-step cycling profiles)
- → **Universal** – reliable detection of RNA targets from broad range of samples

Applications

- → RT-qPCR
- → gene expression analysis
- → genetic profilling
- → miRNA profiling / quantification
- → mass screening
- → RNA viral pathogen detection
- → characterization of genetically modified organisms (GMO)

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Protocol

1. Prior to use, thaw the reagents completely, mix thoroughly by pipetting or inverting the tube and spin briefly. Avoid direct light during next steps.
2. Prepare the RT-qPCR Master Mix placed on ice or in a freezing rack by combining the following reaction reagents in a sterile nuclease-free tube:

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
2x *AMPLIFYME* Probe RT-qPCR Mix	10 µl	1x
Forward primer (10 µM)	0.8 µl (0.4 µM)	0.2 – 1 µM
Reverse primer (10 µM)	0.8 µl (0.4 µM)	0.2 – 1 µM
Probe (10 µM)	0.2 µl (0.1 µM)	0.05 – 0.4 µM
RNase Inhibitor	0.1 µl	–
Reverse Transcriptase	0.1 µl	–
Template	up to 8 µl	1 pg – 1 µg (Total RNA) from 0.01 pg (mRNA)
Nuclease-free water	fill up to 20 µl	–

TABLE 1. RT-qPCR reaction mixture content

3. Determine the total volume for the appropriate number of reactions, plus 10% overage and prepare the Master Mix of all reagents except for RNA template. Mix the components by pipetting or inverting the tube and spin briefly.
4. Aliquot the contents into qPCR tubes or multiple wells of qPCR reaction plate.
5. Add RNA templates to qPCR tubes/plate.
6. Cap qPCR tubes with optical caps or seal the plate with qPCR foil.
7. Spin qPCR tubes/plate for 1–2 min to remove air bubbles and collect liquid to the bottom of the tube.
8. Transfer qPCR tubes/plate to a thermal cycler block and run RT-qPCR reaction.
9. Program your qPCR instrument with the following conditions:
    1) If possible, select FAST cycling option.
    2) Choose the detection channel of the qPCR instrument that corresponds with the fluorophore used in the assay.
    3) Set a thermal cycling profile according to the table below (note that the following conditions are suitable for amplicons of up to 200 bp and may vary depending on different instrument-specific protocols).

Step	Temperature	Time	Cycle
Reverse transcription*	45°C	900 s	
Activation and denaturation	95°C	180 s	
Denaturation	95°C	5 s	40 cycles
Annealing / Extension / Fluorescence Detection**	60°C	45 s	

TABLE 2. Two-step thermal cycling profile

** The reverse transcription reaction time can be between 10–20 min and temperature can be increased up to 50°C.*

*** The annealing/extension time can be between 30–60 s and temperature can be increased up to 65°C.*

Real-time PCR Instrument Compatibility

Instrument	Product Name
Qiagen Rotor-Gene™ instruments, Bio-Rad® Opticon™, Opticon™ instruments, Chromo 4™, CFX96™, CFX384™, Eppendorf Mastercycler® instruments, Cepheid® SmartCycler®, Roche LightCycler® 480, 96, Nano, 1.5/2.0⋆, Illumina® Eco™, Thermo Piko Real®, TaKaRa Thermal Cycler Dice®, Analytik Jena qTOWER, Techne® Quantica®, PrimeQ and ITS International MyGo®	***AMPLIFY**ME* Probe One-Step No-Rox RT-qPCR Mix or ***AMPLIFY**ME* Probe One-Step Universal RT-qPCR Mix
Applied Biosystems™ 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™ and StepOne™ Plus	***AMPLIFY**ME* Probe One-Step Universal RT-qPCR Mix
Applied Biosystems™ 7500, 7500 Fast, ViiA7™, QuantStudio™ instruments, Agilent MX3000P®, MX3005P®, MX4000P® and Fluidigm BioMark™	***AMPLIFY**ME* Probe One-Step Universal RT-qPCR Mix

TABLE 3. Real-Time PCR instrument compatibility

⋆ *For glass capillaries, non-acetylated BSA should be added to the mixture to a final concentration of 250 ng/µl*

Additional information

- → The ***AMPLIFY****ME* Probe One-Step No-ROX RT-qPCR Mix is compatible with variety of qPCR instrument types that do not require the use of passive reference dye (see table 3). For ROX-dependent instruments use the ***AMPLIFY****ME* Probe One-Step Universal RT-qPCR Mix (AM09.1), which includes additional tubes with High ROX and Low ROX solutions.
- → Acquisition of high quality, intact RNA, free of genomic DNA and RNase traces, is vital for the synthesis of a full-length cDNA followed by an accurate quantitative analysis (qPCR).

  The following recommendations for working with RNA should therefore be followed:
  - → Maintain aseptic working conditions: use disposable gloves, changing them as frequently as required; use RNase-free consumables; work only in an area assigned for working with RNA and with equipment dedicated for that purpose.
  - → DNase enzyme may be used if obtaining a DNA-free RNA sample is required.
- → Special attention should be also paid to PCR products from previous reactions since they represent the greatest danger of contamination. In order to prevent carry-over DNA contamination, it is recommended that the RT-qPCR reaction set-up, PCR amplification and any post-PCR analysis should be carried out in separate areas with the use of separate pippets. It is very important that any tubes containing amplified PCR products are not opened in the PCR set-up area.

⚠ **While analysing similarities between the sensitivity of *AMPLIFY****ME* Mixes with competitors' mixes, it is highly advisable to carry out the amplification process with a 10-fold template dilution series. Loss of signal for low copy targets is the only, distinct survey of sensitivity. Please note that an early Ct value is a determinant of the amplification speed, but not its sensitivity.**

- → RNA contamination with genomic DNA may have an influence on data reliability. Therefore no reverse transcription control should be prepared, by omitting reverse transcriptase in reaction content.
- → The usage of intron-spanning primers is strongly recommended to avoid amplification of genomic DNA (common DNA contamination from RNA extraction steps).

AMPLIFYME Probe One-Step No-ROX RT-qPCR Mix

Components	AM08.1-100 100 rxns (20 µl)	AM08.1-500 500 rxns (20 µl)	AM08.1-S 10 rxns (20 µl)
2x *AMPLIFYME* Probe RT-qPCR Mix	1 ml	5 x 1 ml	100 µl
RNase Inhibitor	10 µl	50 µl	1 µl
Reverse Transcriptase	10 µl	50 µl	1 µl
Nuclease-free water	1.9 ml	2 x 1.9 ml	200 µl

Quality Control

The ***AMPLIFYME*** Probe One-Step No-ROX RT-qPCR Mix is tested for its performance in RT-qPCR assay. Free of nonspecific DNA and RNA nucleases contamination.

Storage & shipping

Storage conditions
Store all components at -20°C. Multiple freeze/thawing is not recommended. Aliquoting can be applied if necessary.

Shipping conditions
Shipping on dry or blue ice.

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