AMPLIFYME

SG One-Step Universal RT-qPCR Mix

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The ***AMPLIFYME*** SG One-Step Universal RT-qPCR Mix is a convenient reaction mixture created for reproducible and efficient first-strand cDNA synthesis and subsequent Real-Time PCR in a single tube.

Ready-to-use, 2x concentrated Mix contains all ingredients necessary for Real-Time PCR based on intercalating dsDNA binding dye chemistry: hot-start Taq polymerase, dNTPs, specially developed buffer, stabilizers and enhancers. Additionally, Mu-MLV Reverse Transcriptase and RNase Inhibitor and ROX solution 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*** SG One-Step Universal 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
- → **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 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* SG RT-qPCR Mix	10 µl	1x
Forward primer (10 µM)	0.8 µl (0.4 µM)	0.1 – 1 µM
Reverse primer (10 µM)	0.8 µl (0.4 µM)	0.1 – 1 µM
50x ROX solution*	0.4 µl	1x
RNase Inhibitor	0.4 µl	–
Reverse Transcriptase	0.2 µl	–
Nuclease-free water	fill up to 16 µl	–
Template	4 µl	1 pg – 1 µg (Total RNA) from 0.01 pg (mRNA)

TABLE 1. ***RT-qPCR reaction mixture content***

** Addition of ROX passive dye depends on the type of Real-Time PCR instrument. Please refer to the table 4 "Real-Time PCR instrument compatibility" and add appropriate volume of 50x High ROX solution, 50x Low ROX solution or omit this reagent.*

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) Select the SYBR® Green or FAM detection channel of the qPCR instrument.
    3) Set a thermal cycling profile according to the one of the table belowe (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 trancription	45°C	600 s	
Activation and denaturation	95°C	120 s	
Denaturation	95°C	5 s	40 cycles
Annealing	60°C	10 s	
Extension / Fluorescence Detection	72°C	5 s	
Melt curve analysis	according to the qPCR instrument manual		

TABLE 2. Three-step thermal cycling profile

Step	Temperature	Time	Cycle
Reverse transcription	45°C	600 s	
Activation and denaturation	95°C	120 s	
Denaturation	95°C	5 s	40 cycles
Annealing / Extension / Fluorescence Detection	60°C	20 s	
Melt curve analysis	according to the qPCR instrument manual		

TABLE 3. Two-step thermal cycling profile

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* SG One-Step No-Rox RT-qPCR Mix or ***AMPLIFY**ME* SG One-Step Universal RT-qPCR Mix
Applied Biosystems™ 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™ and StepOne™ Plus	***AMPLIFY**ME* SG One-Step Universal RT-qPCR Mix
Applied Biosystems™ 7500, 7500 Fast, ViiA7™, QuantStudio™ instruments, Agilent MX3000P®, MX3005P®, MX4000P® and Fluidigm BioMark™	***AMPLIFY**ME* SG One-Step Universal RT-qPCR Mix

TABLE 4. 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 ***AMPLIFYME*** SG One-Step Universal RT-qPCR Mix has been optimized for use with variety of qPCR instrument types, including those that use no passive reference normalization and those that use a low or high concentration of passive reference dye (ROX). The mixture is supplied as a ready-to-use, versatile solution without a passive dye ROX, which is supplied in separate tubes. The list of qPCR instruments that do not require the use of ROX or require it at the appropriate concentration is given in the table 4.
- 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 seperate areas with the use of seperate 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 *AMPLIFYME* 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 influance 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 SG One-Step Universal RT-qPCR Mix

Components	AM07-100 100 rxns (20 µl)	AM07-500 500 rxns (20 µl)	AM07-S 10 rxns (20 µl)
2x *AMPLIFYME* SG RT-qPCR Mix	1 ml	5x 1 ml	100 µl
50x High ROX solution	40 µl	200 µl	4 µl
50x Low ROX solution	40 µl	200 µl	4 µl
RNase Inhibitor	40 µl	200 µl	4 µl
Reverse Transcriptase	20 µl	100 µl	2 µl
Nuclease-free water	700 µl	2x 1.7 ml	70 µl

Quality Control

The ***AMPLIFYME*** SG One-Step Universal RT-qPCR Mix is extensively tested for its performance in different RT-qPCR assays. Free of deoxyribonuclease contamination. Free of DNA contamination.

Storage & shipping

Storage conditions

Store all components at -20°C. Multiple freeze/thawing is not recomended. Aliqoutting can be applied if necessary. ***AMPLIFYME*** SG RT-qPCR Mix and ROX solution should be kept in dark.

Shipping conditions

Shipping on dry or blue ice.

ⓘ For research use only

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