AMPLIFYME

SG No-ROX Mix

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AMPLIFY*ME*
SG No-ROX Mix

The ***AMPLIFY****ME* SG No-ROX Mix is a convenient enzyme mixture for fast and reliable quantitative Real-Time PCR, using intercalating dsDNA-binding dye.

Ready-to-use, 2x concentrated Master Mix contains *TaqNovaHS* polymerase which is a mixture of recombinant *Taq* polymerase over-expressed in *E. coli* and a highly specific monoclonal anti-Taq antibody. The *TaqNovaHS* polymerase enables easy set-up of a hot-start PCR reaction at room temperature. The antibody binds reversibly to the enzyme, inhibiting polymerase activity at ambient temperatures, what prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR set-up. The antibody is released from the polymerase during initial DNA denaturation step, thus providing full DNA polymerase activity during standard cycling conditions.

Precisely optimized buffer components, reliable DNA polymerase and the appropriate selection of PCR enhancers provide a high performance, sensitivity and specificity over a broad dynamic range and give 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 DNA or cDNA targets
- → **Reproducible** – consistent amplification across a wide dynamic range (6 logs)
- → **Specific** – precisely selected anti-Taq antibody eliminates non-specific amplification (tested up to 45 cycles)
- → **Fast** – accurate detection of molecular targets in as fast as 30 minutes (with either two- or three-step cycling profiles)
- → **Stable** – no loss of activity after 8 successive freeze/thaw cycles

Applications

- → qPCR / Real-Time PCR
- → gene expression analysis
- → mass screening
- → pathogen detection
- → characterization of genetically modified organisms (GMO)
- → copy number variation analysis

Formulation

2x concentrated mix: *TaqNovaHS* polymerase, dNTPs, 6 mM $MgCl_2$, PCR enhancers, stabilizers, optimized buffer.

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Protocol

1. Prior to use, thaw the reagents completely, mix thoroughly by pipetting or vortexing and spin briefly. Avoid direct light during next steps.
2. Prepare the 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 Mix	10 µl	1x
Forward primer (10 µM)	0.6 µl (0.3 µM)	0.1 – 0.8 µM
Reverse primer (10 µM)	0.6 µl (0.3 µM)	0.1 – 0.8 µM
DNA or cDNA template	1 – 100 ng	1 pg – 0.5 µg
PCR-grade water	fill up to 20 µl	

TABLE 1. Real-Time PCR 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 DNA 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 DNA templates to qPCR tubes/plate.
6. Seal the plate with qPCR foil or cap qPCR tubes with optical caps.
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 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 tables below (note that the following conditions are suitable for amplicons of up to 250 bp and may vary depending on different instrument-specific protocols).

Step	Temperature	Time	Cycle
Activation and denaturation	95°C	180 s	
Denaturation	95°C	5 s	35–45 cycles
Annealing	60°C	10 s	
Extension / Fluorescence Detection	72°C	5–20 s	
Melt curve analysis	according to the qPCR instrument manual		

TABLE 2. Three-step thermal cycling profile

Step	Temperature	Time	Cycle
Activation and denaturation	95°C	180 s	
Denaturation	95°C	5 s	35–45 cycles
Annealing / Extension / Fluorescence Detection	60°C	15–30 s*	
Melt curve analysis	according to the qPCR instrument manual		

TABLE 3. Two-step thermal cycling profile

** It is not recommended to use annealing/extension times longer than 30 seconds.*

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®	***AMPLIFYME*** SG No-ROX Mix or ***AMPLIFYME*** SG Universal Mix
Applied Biosystems™ 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™ and StepOne™ Plus	***AMPLIFYME*** SG Universal Mix
Applied Biosystems™ 7500, 7500 Fast, ViiA7™, QuantStudio™ instruments, Agilent MX3000P®, MX3005P®, MX4000P® and Fluidigm BioMark™	***AMPLIFYME*** SG Universal 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 ***AMPLIFY****ME* SG No-ROX Mix is compatible with variety of qPCR instrument types that do not require the use of passive reference dye (see table 4). For ROX-dependent instruments use the ***AMPLIFY****ME* SG Universal Mix (AM02), which includes additional tubes with High ROX and Low ROX solutions.
- The mixture's extreme sensitivity means that it is highly susceptible to DNA contamination. Therefore, disposable gloves should be worn at all times. 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 preparing and portioning of the qPCR Master Mix, addition of DNA template, DNA amplification and any post-PCR analysis are carried out in separate areas with the use of separate pipettes. 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.**

- Always include a non-template control reaction, replacing DNA or cDNA with PCR-grade water.
- When amplifying cDNA, the usage of intron-spanning primers is strongly recommended to avoid amplification of genomic DNA (common DNA contamination from RNA extraction steps).

AMPLIFYME SG No-ROX Mix

Components	AM01-020 200 rxns (20 µl)	AM01-200 2000 rxns (20 µl)	AM01-S 20 rxns (20 µl)
2x *AMPLIFYME* SG Mix	2 x 1 ml	20 x 1 ml	200 µl
PCR-grade water	2 x 1.5 ml	20 x 1.5 ml	300 µl

Quality Control

The ***AMPLIFYME*** SG No-ROX Mix is extensively tested for its performance in different Real-Time PCR assays. Free of deoxyribonuclease contamination.

Storage & shipping

Storage conditions
Long-term storage: -20°C, after thawing store at 2-8°C for up to 1 month. The ***AMPLIFYME*** SG No-ROX Mix does not lose its activity after eight successive freeze/thaw cycles. Keep in dark.

Shipping conditions
Shipping on dry or blue ice

For research use only

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