August 2023

Quick-Start Protocol

qPCR SG Mix ROX

The qPCR SG Mix ROX (cat. nos. AM02-020 and AM02-200) is an enzyme mixture for quantitative Real-Time PCR, using intercalating dsDNA-binding dye.

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

The qPCR SG Mix ROX ships on dry ice. Storage in the long term is at –20°C and after thawing at 2–8°C for two months. It does not lose its activity after eight successive freeze/thaw cycles.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The qPCR SG Mix ROX 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 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.
  Important: While analysing the sensitivity of qPCR SG 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)

Procedure

1. Thaw the reagents completely, mix thoroughly by pipetting or vortexing and spin briefly.
   Note: Avoid direct light during the next steps.
2. Prepare the qPCR Master Mix by combining the following reaction reagents in a sterile nuclease-free tube:

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.

Table 1.Real-Time PCR reaction mixture content

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
2x qPCR 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
50x ROX solution*	0.4 µL	1x
DNA or cDNA template	1 – 100 ng	1 pg – 0.5 µg
PCR-grade water	fill up to 20 µL	

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

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:
   - If possible, select FAST cycling option.
   - Select the SYBR® Green or FAM detection channel of the qPCR instrument.
   - 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)

Table 2. Three-step thermal cycling profile

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

Table 3. Two-step thermal cycling profile

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

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

Document Revision History

Date	Changes
08/2023	Initial release

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