EURx® MOLECULAR BIOLOGY PRODUCTS

Fast SG/ROX qPCR Master Mix (2x)

Kit Components

Component	Cat. No. E0413-01 100 reactions of 25 µl	Cat. No. E0413-02 200 reactions of 25 µl	Cat. No. E0413-03 1000 reactions of 25 µl
Fast SG/ROX qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	30 µl	55 µl	270 µl
Water, nuclease free	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml

Storage

Store at –20°C in the dark for long-term storage or at 4°C for up to 1 month.

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Description

- Fast SG/ROX qPCR Master Mix (2x) is a universal solution for fast-cycling quantitative real-time PCR and two-step real-time RT-PCR and can be used on most real-time PCR cyclers available regardless of requirements in ROX reference dye.
- The master mix contains Perpetual Taq DNA Polymerase, optimized reaction buffer, dNTPs (dTTP is partially replaced with dUTP), SYBR Green I dye and ROX passive reference dye.
- SYBR Green I enables DNA analysis without having to synthesize expensive sequence-specific labeled probes.
- Perpetual Taq DNA Polymerase contains recombinant Taq DNA Polymerase bound to anti-Taq monoclonal antibodies that block polymerase activity at moderate temperatures.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for at least two minutes.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup leading to higher specificity and sensitivity of PCR reactions.
- The polymerase enables convenient room temperature reaction setup.
- SYBR Green I is a fluorescent dye which binds all double-stranded DNA molecules and emits a fluorescent signal on binding. The excitation and emission maxima of SYBR Green I are at 494 nm and 521 nm, respectively, which are compatible with use on any real-time cycler.
- Fast SG/ROX qPCR Master Mix (2x) contains dUTP, which partially replaces dTTP. It allows the optional use of a uracil-N-glycosylase (UNG) to prevent carryover contamination between reactions. UNG removes uracil from any dU-containing contaminating amplicons, leaving abasic sites and making DNA molecules susceptible to hydrolysis during the initial denaturation step.
- ROX passive reference dye included in the master mix allows fluorescence normalization on certain cyclers. The use of ROX dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Agilent. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction, has a different emission spectrum than SYBR Green I and does not interfere with real-time PCR on any instrument.
- Fast SG/ROX qPCR Master Mix (2x) is intended to use with most cyclers from Applied Biosystems, Bio-Rad, Cepheid, Roche, Eppendorf, Agilent, Qiagen, etc.

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Protocol

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
Fast SG/ROX qPCR Master Mix (2x)	12.5 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.5 µM
Reverse Primer	Variable	0.5 µM
Template DNA	Variable	≤500 ng
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	0.25 µl	0.25 U/reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. Minimize exposure of Fast SG/ROX qPCR Master Mix (2x) to light during handling to avoid loss of fluorescent signal intensity.
2. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
3. Optimal amplicon length in real-time PCR using SYBR Green I is 70-200 bp.
4. Thaw, gently vortex and briefly centrifuge all solutions.
5. Set up PCR reactions at room temperature. Use of Fast SG/ROX qPCR Master Mix (2x) allows room temperature reaction setup.
6. Prepare a reaction master mix by adding all the reaction components except template DNA.
7. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
8. Add template DNA/cDNA (≤500 ng/reaction) to the individual PCR tubes or wells containing the reaction mix. For two-step RT-PCR, the volume of cDNA added should not exceed 10% of the final PCR volume.
9. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
10. Place the samples in the cycler and start the program.
11. The concentration of $MgCl_2$ in 1 x Fast SG/ROX qPCR Master Mix is 2.5 mM. In most cases this concentration will produce optimal results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to a reaction.
12. The recommended starting concentration of primers is 0.5 µM. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used. Raising primer concentration may increase PCR efficiency, but negatively affect PCR specificity. If nonspecific products are generated decrease primers concentration up to 0.3 µM.
13. Readjust the threshold value for analysis of every run.
14. If using Bio-Rad iCycler iQ or MyiQ instruments collect well factors at the beginning of each experiment. Use an external well factor plate according to the manufacturer's recommendations. Well factors are used to compensate for any excitation or pipetting variations.

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Thermal Cycling Conditions:

2-step cycling

Step	Temperature	Time	Number of Cycles
Optional: Thermolabile UNG pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	2-5 min	1
Denaturation	95°C	10 s	35-45
Annealing/Extension	60°C	30 s	
Cooling	4°C	Indefinite	1

3-step cycling

Step	Temperature	Time	Number of Cycles
Optional: Thermolabile UNG pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	2-5 min	1
Denaturation	95°C	10 s	35-45
Annealing/	50–65°C	10 s	
Extension	72°C	15 s	
Cooling	4°C	Indefinite	1

Notes:

1. Fast SG/ROX qPCR Master Mix (2x) has been developed for use in a two-step cycling protocol. This protocol works well for most primers (even for primers with a T_m well below 60°C).
2. The incubation step of 37°C for 2 minutes must be added if a thermolabile uracil-N-glycosylase is used to prevent carryover contamination. UNG degrades any dUMP-containing PCR products.
3. During the initial denaturation step thermolabile UNG and antibodies that block Taq DNA Polymerase are inactivated.
4. Melting curve analysis should be performed to verify the specificity and identity of PCR products. Melting curve analysis is an analysis step built into the software of real-time cyclers. Melting curve data between 65°C and 95°C should be acquired.
5. Always check the PCR product specificity by gel electrophoresis when designing a new assay. Melting temperatures of the specific product and primer-dimers may overlap.

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