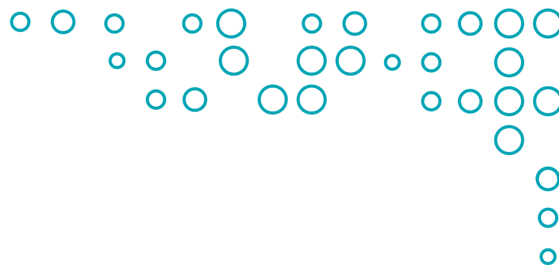
EURx® MOLECULAR BIOLOGY PRODUCTS



Inhibitor-Tolerant SG qPCR Master Mix (2x)

Kit Components

Component	Cat. No. E0410-01 200 reactions of 20 µl	Cat. No. E0410-02 500 reactions of 20 µl	Cat. No. E0410-03 1000 reactions of 20 µl
Inhibitor-Tolerant SG qPCR Master Mix (2x)	2 x 1 ml	5 x 1 ml	10 x 1 ml
Water, nuclease free	2 x 1 ml	5 x 1 ml	10 x 1 ml

Storage

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

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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Description

- Inhibitor-Tolerant SG qPCR Master Mix (2x) is a universal solution optimized for challenging real-time PCR and two-step real-time RT-PCR and can be used on most real-time PCR cyclers available regardless of requirements for ROX reference dye.
- The master mix enables reactions with a range of DNA samples, such as: purified DNA, lysates, tissue extracts, undiluted cDNA, samples containing inhibitors including heparin, hematin, polysaccharides, polyphenols, salts, humic acid, cell culture media, etc.
- The master mix contains onHybrid DNA Polymerase, optimized reaction buffer, dNTPs, SYBR Green I dye and a blend of passive reference dyes (ROX and fluorescein).
- SYBR Green I enables DNA analysis without having to synthesize expensive sequence-specific labeled probes.
- onHybrid DNA Polymerase is a modified hot start enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 98°C for at least 10 minutes.
- 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.
- Inhibitor-Tolerant SG qPCR Master Mix (2x) is intended for 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
Inhibitor-Tolerant SG qPCR Master Mix (2x)	10 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.2-0.5 µM
Reverse Primer	Variable	0.2-0.5 µM
Template DNA	Variable	≤500 ng
Water, nuclease free	To 20 µl	-
Total volume	20 µl	-

Notes:

1. Minimize exposure of Inhibitor-Tolerant SG qPCR Master Mix (2x) to light during handling to avoid loss of fluorescent signal intensity.
2. A reaction volume of 20 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 Inhibitor-Tolerant SG 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. The volume of undiluted cDNA, cell lysate/tissue extracts added should not exceed 20% 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 recommended starting concentration of primers is 0.4 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 primer concentration up to 0.2 µM.
12. Readjust the threshold value for analysis of every run.
13. 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:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	98°C	10 min	1
Denaturation	98°C	5-10 s	35-45
Annealing/Extension	60°C	45 s	
Cooling	4°C	Indefinite	1

Notes:

1. During the initial denaturation step onHybrid DNA Polymerase is activated. onHybrid DNA Polymerase requires 10 min incubation at 98°C to be activated.
2. Inhibitor-Tolerant SG 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). Annealing temperature can be adjusted if necessary.
3. The optimal time of the annealing/extension step is usually 45 s. If necessary, the duration of the annealing/extension step can be adjusted in the range of 30-60 s.
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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