EURx® MOLECULAR BIOLOGY PRODUCTS

SG onTaq qPCR Master Mix (2x)

Kit Components

Component	Cat. No. E0405-01 100 reactions of 25 µl	Cat. No. E0405-02 200 reactions of 25 µl	Cat. No. E0405-03 1000 reactions of 25 µl
SG onTaq qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
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

SG onTaq qPCR Master Mix (2x), plus ROX Solution

Kit Components

Component	Cat. No. E0406-01 100 reactions of 25 µl	Cat. No. E0406-02 200 reactions of 25 µl	Cat. No. E0406-03 1000 reactions of 25 µl
SG onTaq qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
ROX Solution, 25 µM	55 µl	110 µl	530 µl
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 2 weeks.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Description

- SG onTaq qPCR Master Mix (2x) is a universal solution for quantitative real-time PCR and two-step real-time RT-PCR and can be used on most real-time PCR cyclers available.
- The master mix contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs (dTTP is partially replaced with dUTP) and SYBR Green I dye.
- SYBR Green I enables DNA analysis without having to synthesize expensive sequence-specific labeled probes.
- onTaq 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 15 min initial denaturation step at 95°C.
- 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.
- SG onTaq 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.
- There are two variants of the kit: without ROX and with ROX Solution provided separately. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. 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. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 25 µl reaction	Amount of ROX per 1.25 ml of 2x master mix	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM	0.5 µl	50 µl	500 nM
Applied Biosystems: 7500, ViiA 7, Stratagene: Mx3000P, Mx3005P,	0.5 µl 10 x diluted (in water)	50 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	Not required	-

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Protocol

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
SG onTaq qPCR Master Mix (2x)	12.5 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.3-0.5 µM
Reverse Primer	Variable	0.3-0.5 µM
Template DNA	Variable	≤500 ng
Optional: ROX Solution, 25 µM	0.5 µl or 0.5 µl 10 x diluted	500 nM 50 nM
Optional: 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 SG onTaq qPCR Master Mix (2x) and ROX 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 if needed, gently vortex and briefly centrifuge all solutions.
5. Set up PCR reactions at room temperature. Use of SG onTaq 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. Standard concentration of $MgCl_2$ in real-time PCR reaction is 2.5 mM (as provided with the 1 x SG onTaq qPCR Master Mix). 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. A final primer concentration of 0.3-0.5 µM is usually optimal, but can be individually optimized in range of 0.1 µM to 1 µM. The recommended starting concentration is 0.5 µM. Raising primer concentration may increase PCR efficiency, but negatively affect PCR specificity. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
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:

Step	Temperature	Time	Number of Cycles
Optional: UNG pre-treatment	50°C	2 min	1
Initial Denaturation	95°C	15 min	1
Denaturation Annealing Extension Optional: Data acquisition	94°C 50-60°C 72°C X°C	15 s 30 s 30 s 15 s	35-45
Cooling	4°C	Indefinite	1

Notes:

1. The incubation step of 50°C for 2 minutes must be added if a uracil-N-glycosylase is used to prevent carryover contamination. UNG degrades any dUMP-containing PCR products.
2. During the initial denaturation step onTaq DNA Polymerase is activated and UNG is inactivated. onTaq DNA polymerase requires 15 min incubation at 95°C to be activated.
3. UNG activity may be partially restored at temperatures lower than 55°C due to refolding. It is recommended to perform PCR using a temperature equal 55°C or above for the annealing step. After completing the PCR, cool reactions to 4°C and load directly on a gel or store frozen.
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. Data acquisition should be performed during the extension step. To suppress fluorescence readings caused by the generation of primer-dimers an additional data acquisition step can be added to the protocol. It is possible when T_m of primer-dimers differs from T_m of the specific product (T_m are generated during melting curve analysis). The temperature of the data acquisition step should be above T_m of primer-dimers but approximately 3°C below the T_m of the specific product.
6. 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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