

SG 1-Step RT-qPCR Master Mix + ROX

ONE STEP REAL TIME RT-PCR KIT



Kit Components:

- The **Master Enzyme Mix** contains a unique highly sensitive reverse transcriptase, Hot Start Perpetual Taq DNA Polymerase, RNase Inhibitor and SYBR Green I dye, respectively.
- qRT-PCR Master Buffer (2x)** is a universal reaction buffer with dNTPs (dTTP is partially replaced with dUTP) that can be used with most real-time PCR cyclers available.
- Thermolabile Uracil-N-Glycosylase (UNG)**,
- nuclease-free water**.

SG OneStep qRT-PCR kit is a one-step qRT-PCR kit that provides accurate real-time quantification of RNA targets. The kit is composed of a unique reverse transcriptase and a highly processive hot start Perpetual Taq DNA Polymerase in an easy to use format.

Description:

- The reverse transcriptase works in a broad range of temperatures between 25 and 55°C without any loss of specificity and sensitivity.
- Both, cDNA synthesis and PCR, are performed in one single tube using gene-specific primers and either total RNA or mRNA as template.
- Perpetual *Taq* DNA Polymerase contains a recombinant *Taq* DNA Polymerase bound to anti-*Taq* monoclonal antibodies which blocks 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.
- 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 1-Step RT-qPCR Master Mix contains dUTP, which partially replaces dTTP. It allows the optional use of a thermolabile 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 mandatory for all real-time PCR cyclers from Applied Biosystems and is 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 specific PCR cyclers.

SG qPCR Master Mix (2x)

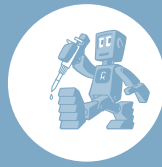
Component	Cat. No. E0810-01	Cat. No. E0810-02
	25 reactions, 25 µl each, 625 µl [1x] final volume	100 reactions, 25 µl each, 2.5 ml [1x] final volume
SG 1-Step RT-qPCR Master Enzyme Mix	25 µl	100 µl
qRT-PCR Master Buffer (2x)	1 x 350 µl	2 x 0.7 ml
Thermolabile UNG (Uracil-N-Glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 0,5 ml	2 x 1.25 ml

SG qPCR Master Mix (2x) plus ROX Solution

Component	Cat. No. E0811-01	Cat. No. E0811-02
	100 reactions, 25 µl each, 2.5 ml [1x] final volume	200 reactions, 25 µl each, 5 ml [1x] final volume
SG 1-Step RT-qPCR Master Enzyme Mix	25 µl	100 µl
qRT-PCR Master Buffer (2x)	1 x 350 µl	2 x 0.7 ml
ROX Solution, 25 µM	25 µl	100 µl
Thermolabile UNG (Uracil-N-Glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 1.25 ml	2 x 1 ml

Storage:

Store at -20°C in the dark.



SG 1-Step RT-qPCR Master Mix

REAL TIME PCR PROTOCOL

qPCR- Protocol

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

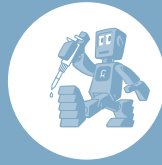
Instrument	Amount of ROX per 25 μ l reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.3-0.5 μ l	300-500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.3-0.5 μ l 10 x diluted (in water)	30-50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

Preparation of PCR Reaction:

Component	Volume/Reaction	Final Concentration
qRT-PCR Master Buffer (2x)	12.5 μ l	1 x 3 mM MgCl ₂
Forward Primer	Variable	0.3 - 0.5 μ M
Reverse Primer	Variable	0.3 - 0.5 μ M
Template RNA	Variable	500 ng
Optional: ROX Solution, 25 μ M	0.3-0.5 μ l or 0.3-0.5 μ l 10 x diluted	300-500 nM 30-50 nM
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/ μ l	0.25 μ l	0.25 U / reaction
SG 1-Step RT-qPCR Master Enzyme Mix	1 μ l	1 μ l / reaction
Water, nuclease free	To 25 μ l	-
Total volume	25 μ l	-

Notes:

- Minimize freeze-thaw cycles** of qRT-PCR Master Buffer (2x). Always keep Master Enzyme Mix and ROX solution on ice and minimize light exposure during handling to avoid losses in fluorescent signal intensity.
- Concentration Differences.** Completely thaw and gently mix qRT-PCR Master Buffer (2x) before use.
- Reaction Volume.** A reaction volume of 25 μ l is recommended for most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
- Amplicon Length.** The optimal amplicon length in real-time PCR using SYBR Green I is 70-200 bp.
- Exon-Exon Primers.** To avoid amplification from contaminating genomic DNA, design exon-exon primers. For primers designed for *H.sapiens* RNA, free online software is available: <http://primerdepot.nci.nih.gov/>
- Setup On Ice.** Set up RT- PCR reactions on ice to minimize any RNA template degradation.
- Prepare a reaction master mix** by adding all the reaction components except template RNA. The RNA template (500 ng/reaction) should be added to the individual PCR tubes or wells containing the whole reaction mix. Centrifuge briefly before placing the reactions into the cycler. Check if there remain no residual air bubbles. In case of any remaining air bubbles, repeat the centrifugation step. Air bubbles interfere with fluorescent detection.
- Start.** Place the samples in the cycler and start the program.
- RT Temperature Range.** The reverse transcriptase works in a broad range of temperatures between 25°C and 55°C. The recommended starting temperature for reverse transcription is 50 °C. For individual experimental requirements, the RT incubation temperature might be changed.
- MgCl₂.** The standard concentration of MgCl₂ in real-time PCR reactions is 3.0 mM (as provided with the qRT-PCR Master buffer). In most cases this concentration will yield optimal results. However, if higher MgCl₂ concentrations are required, prepare a 25 mM MgCl₂ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 μ l of a 25 mM MgCl₂ solution to a total reaction volume of 25 μ l will add 25 nmol MgCl₂ and thus increase total MgCl₂ reaction concentration in 1.0 mM.
- Primer Concentration.** A final primer concentration of 0.3-0.5 μ M is usually optimal, but can be individually optimized in a range of 0.1 μ M to 1 μ M. The recommended starting concentration is 0.4 μ M. Raising primer concentration may increase PCR efficiency, but negatively affects PCR specificity. The optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
- Readjust the threshold value** for analysis of every run.
- Well Factors.** When using a 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.



SG 1-Step RT-qPCR Master Mix

REALTIME PCR PROTOCOL

qPCR- Protocol - Thermal Cycling Conditions

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Reverse Transcription	50°C	30 min	1
Initial Denaturation	95°C	3 min	1
Denaturation	94°C	15 s	35-45
Annealing	50-60°C	30 s	
Extension	72°C	20 s	
Optional: Data acquisition	X°C (see Note 5)	15 s	
Cooling	4°C	Infinite	1

Notes:

- Thermolabile Uracil-N-Glycosylase (UNG).** During the reverse transcription step at 50°C, thermolabile uracil-N-glycosylase might be used for protection of carryover contamination (e.g. by aerosol formation) with Uracil-labelled qPCR products from previously performed analyses. Don't use UNG from *E.coli*, which may degrade any newly synthesized cDNA.
- Thermal Inactivation.** During the initial denaturation step UNG and antibodies that block *Taq* DNA Polymerase are inactivated. The anti-*Taq* antibodies and UNG require at least 2 min or 10 min incubation at 95°C, respectively. When UNG is not used within the PCR reaction, the duration of the initial denaturation step can be reduced to 2-5 min at 95°C.
- 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.
- Primer Dimers and Data Acquisition.** Data acquisition should be performed during the extension step. To suppress fluorescence readings caused by the generation of primer-dimers, it is possible to add an additional data acquisition step to the protocol. Discrimination between fluorescence readings induced by PCR product formation from primer-dimer accumulation is possible, if the T_m of primer-dimers is lower than the T_m of specific PCR products (T_m are determined during melting curve analysis). For this reason, choose a suitable temperature for the data acquisition step well above the T_m of primer-dimers, but approximately 3°C below the T_m of the specific product.
- QC by Agarose Gel Electrophoresis.** 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.