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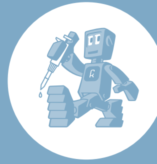

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SG 1-Step RT-qPCR Master Mix

ONE STEP REAL TIME RT-PCR KIT

Kit Components:

- The **Master Enzyme Mix** contains a unique highly sensitive reverse transcriptase, Hot Start onTaq DNA Polymerase, RNase Inhibitor and SYBR Green I dye, respectively.
- **2x RT-PCR SG Buffer** 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 qPCR Master Mix (2x)

Component	**Cat. No.** E0810-01 25 reactions, 25 µl each, 625 µl [1x] final volume	**Cat. No.** E0810-02 100 reactions, 25 µl each, 2.5 ml [1x] final volume
SG Enzyme Mix	25 µl	100 µl
2x RT-qPCR SG Buffer	1 x 350 µl	2 x 700 µl
Thermolabile UNG (Uracil-N-Glycosylase) 1 U/µl	10 µl	30 µl
RNase-free Water	1 x 0.5 ml	2 x 1.0 ml

SG qPCR Master Mix (2x) plus ROX Solution

Component	**Cat. No.** E0811-01 25 reactions, 25 µl each, 625 µl [1x] final volume	**Cat. No.** E0811-02 100 reactions, 25 µl each, 2.5 ml [1x] final volume
SG Enzyme Mix	25 µl	100 µl
2x RT-qPCR SG Buffer	1 x 350 µl	2 x 700 µl
ROX Solution, 25 µM	15 µl	60 µl
Thermolabile UNG (Uracil-N-Glycosylase) 1 U/µl	10 µl	30 µl
RNase-free Water	1 x 0.5 ml	2 x 1 ml

Storage:
Store at -20°C in the dark.

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, highly processive "hot start" onTaq DNA Polymerase, Ribonuclease Inhibitor and - for optional use – thermolabile Uracil N-Glycosylase in an easy to use format.

Description:

- The unique reverse transcriptase works in a broad range of temperatures between 35°C and 55°C without any loss of specificity and sensitivity.
- 2 x RT-qPCR SG Buffer is a universal reaction buffer with dNTPs (dTTP is partially replaced with dUTP). The assay is compatible with most real-time PCR cyclers available and allows optional use of thermolabile uracil-N-glycosylase (UNG) for carryover contamination protection.
- The kit contains thermolabile uracil-N-glycosylase (UNG) that is optimized for RT-qPCR reactions.
- Both, cDNA synthesis and PCR, are performed in one single tube using gene-specific primers and either total RNA or mRNA as template.
- onTaq DNA Polymerase is a genetically engineered "hot start" enzyme, providing extremely "tight" inhibition of DNA polymerase activity at moderate temperatures and during the Reverse Transcription step. The polymerase activity is restored during the initial denaturation step, when amplification reactions are heated at 95°C for at least ten 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.
- 2x RT-qPCR SG Buffer 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 reactions, 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.

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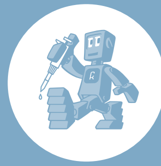

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SG 1-Step RT-qPCR Master Mix

REAL TIME RT-qPCR PROTOCOL (1)

qPCR- Protocol

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

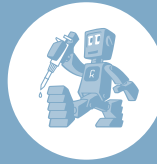
Instrument	Amount of ROX per 25 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.5 µl	500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.5 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad,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

Preparation of PCR Reaction:

Component	Volume/Reaction	Final Concentration
2x RT-qPCR SG Buffer	12.5 µl	1 x 3 mM $MgCl_2$
Forward Primer	Variable	0.4 µM
Reverse Primer	Variable	0.4 µM
Template RNA	Variable	maximum 500 ng
Optional: ROX Solution, 25 µM	0.5 µl or 0.5 µl 10 x diluted	500 nM 50 nM
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	0.25 µl	0.25 U / reaction
SG Enzyme Mix	1 µl	1 µl / reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. **Minimize freeze-thaw cycles** of 2x RT-qPCR SG Buffer. Always keep Master Enzyme Mix and ROX solution on ice and minimize light exposure during handling to avoid losses in fluorescent signal intensity.
2. **Concentration Differences.** Completely thaw and gently mix 2x RT-qPCR SG Buffer before use.
3. **Reaction Volume.** A reaction volume of 25 µl is recommended for most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
4. **Amplicon Length.** The optimal amplicon length in real-time PCR using SYBR Green I is 70-150 bp.
5. **Exon-Exon Primers.** To avoid amplification from contaminating genomic DNA, design exon-exon primers.
6. **Setup On Ice.** Set up RT- PCR reactions on ice to minimize any RNA template degradation.
7. **Prepare a reaction master mix** by adding all the reaction components except template RNA. The RNA template (recommended range: 1 pg to 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.
8. **Start.** Place the samples in the cycler and start the program.
9. **RT Temperature Range**. The reverse transcriptase works in a broad range of temperatures between 35°C and 55°C. The recommended starting temperature for reverse transcription is 50°C. For individual experimental requirements, the RT incubation temperature temperature might be changed.
10. **$MgCl_2$.** The standard concentration of $MgCl_2$ 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 $MgCl_2$ concentrations are required, prepare a 25 mM $MgCl_2$ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 25 µl will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 1.0 mM.
11. **Primer Concentration.** A final primer concentration of 0.3-0.5 µM is usually optimal, but can be individually optimized in a range of 0.1 µM to 1 µM. The recommended starting concentration is 0.4 µM. 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.
12. **Readjust the threshold value** for analysis of every run.
13. **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 RT-qPCR PROTOCOL (2)

qPCR- Protocol - Thermal Cycling Conditions

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Reverse Transcription	50°C	20 min	1
Initial Denaturation	95°C	15 min	1
Denaturation Annealing / Extension / Data acquisition	94°C 60°C	15 s 60 s	40-45
Cooling	4°C	Infinite	1

Notes:

1. **Thermolabile Uracil-N-Glycosylase (UNG).** During the reverse transcription step at 50°C, thermolabile uracil-N-glycosylase might be used for protection from carryover contamination. Carryover contamination is caused by aerosol-borne transfer of Uracil-labelled qPCR products from previously performed analyses. Aerosols are often formed through opening and closing of post-PCR tubes. Don't use UNG from *E.coli*, which may degrade any newly synthesized cDNA.
2. **Thermal Inactivation.** Thermolabile UNG is inactvated at 50°C during the reverse transcription step.
3. **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.
4. **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.
5. **QC by Agarose Gel Electrophoresis.** Always check the PCR product specificity by gel electrophoresis when designing a new assay. Take into consideration that melting temperatures of the specific product and of primer-dimers might eventually overlap.