SG OneStep qRT-PCR kit

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

SG OneStep qRT-PCR kit

Components

Component	Cat. No. E0810-01 25 reactions of 25 µl	Cat. No. E0810-02 100 reactions of 25 µl
qRT-PCR Master Buffer (2x)	1 x 350 µl	2 x 0.7 ml
Master Enzyme Mix	25 µl	100 µl
Thermolabile Uracil-N-glycosylase (UNG) 1U/µl	10 µl	30 µl
Water, nuclease free	1 x 0.5 ml	2 x 1 ml

SG OneStep qRT-PCR kit plus ROX Solution

Components

Component	Cat. No. E0811-01 25 reactions of 25 µl	Cat. No. E0811-02 100 reactions of 25 µl
qRT-PCR Master Buffer (2x)	1 x 350 µl	2 x 0.7 ml
ROX Solution, 25 µM	25 µl	100 µl
Master Enzyme Mix	25 µl	100 µl
Thermolabile Uracil-N-glycosylase (UNG) 1U/µl	10 µl	30 µl
Water, nuclease free	1 x 0.5 ml	2 x 1 ml

Storage

Store at -20°C in the dark.

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This kit provides:

1. qRT-PCR Master Buffer (2x) that is a universal reaction buffer with dNTPs (dTTP is partially replaced with dUTP) that can be used on most real-time PCR cyclers available.
2. The Master Enzyme Mix contains unique highly sensitive reverse transcriptase, Hot Start Perpetual Taq DNA Polymerase, RNAse Inhibitor and SYBR Green I dye.
3. Reverse transcriptase works in high range of temperatures from 25-55°C without loss of specificity and sensitivity.
4. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA.
5. Perpetual Taq DNA Polymerase contains recombinant Taq DNA Polymerase bound to anti-Taq monoclonal antibodies that block polymerase activity at moderate temperatures.
6. The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for at least two minutes.
7. 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.
8. Kit is provided with dUTP that allows the optional use of thermolabile a uracil-N-glycosylase (UNG).
9. If cyclers from Applied Biosystems are used ROX passive reference dye is necessary. **SG OneStep qRT-PCR kit** is provided in two variants: without ROX and with ROX solution provided separately. The table below shows 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	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.3-0.5 µl	300-500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.3-0.5 µl 10 x diluted (in water)	30-50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

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Protocol

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
qRT-PCR Master Buffer (2x)	12.5 µl	1 x
Forward Primer	Variable	0.3-0.5 µM
Reverse Primer	Variable	0.3-0.5 µM
Template RNA	Variable	≤500 ng
Master Enzyme Mix	1 µl	1 µl /reaction
Optional: ROX Solution, 25 µM	0.3-0.5 µl or 0.3-0.5 µl 10 x diluted	300-500 nM 30-50 nM
Optional: thermo labile 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 thaw-freeze cycles of qRT-PCR Master Buffer (2x), keep Master Enzyme Mix and ROX solution on ice and minimize light exposure during handling to avoid loss of fluorescent signal intensity.
2. qRT-PCR Master Buffer (2x) thaw and gently vortex before use.
3. 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.
4. Optimal amplicon length in real-time RT-PCR using SYBR Green I is 70-200 bp.
5. To avoid amplification from genomic DNA design egzon-egzon primers. For primers designed to *H.sapiens* RNA free online software might be used: http://primerdepot.nci.nih.gov/
6. Set up RT- PCR reactions on ice to minimize RNA template degradation.
7. The RNA template (≤500 ng/reaction) should be added to the individual PCR tubes or wells containing the whole reaction mix and centrifuge briefly before placing into cycler. Check if there are not any bubbles left, if yes, spin again.
8. Place the samples in the cycler and start the program.
9. Reverse transcriptase works in wide range of temperatures 25-55°C. The recommended starting temperature for reverse transcription is 50 °C. For individual experiment temperature might be changed.
10. Standard concentration of $MgCl_2$ in real-time RT-PCR reaction is 3 mM (as provided with the 1 x qRT-PCR Master buffer) 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 reaction.
11. 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 0.4 µM. Raising primer concentration may increase PCR efficiency, but negatively affect RT-PCR specificity. 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. 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
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	15 s	
Cooling	4°C	Indefinite	1

Notes:

1. During reverse transcription step of 50°C also thermolabile uracil-N-glycosylase might be used. Don't use UNG from *E.coli,* UNG will degrade all newly synthetized cDNA.
2. Thermolabile UNG is inactvated at 50°C during RT and antibodies that block Taq DNA Polymerase are destryed during 3 minutes of initial denaturation step.
   The anti-Taq antibodies and UNG require at least 2-5 min incubation at 95°C. When UNG is not used in PCR reaction the duration of the initial denaturation step can be reduced to 2 min at 95°C.
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. 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.
5. Always check the RT- 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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