EURx® MOLECULAR BIOLOGY PRODUCTS

SG OneStep PRO RT-qPCR kit

SG OneStep PRO RT-qPCR kit is one-step RT-qPCR kit that provides accurate real-time quantification of RNA targets. The kit consists of unique, proofreading thermostable reverse transcriptase and DNA Polymerase in an easy-to-use format.

SG OneStep PRO RT-qPCR kit

Kit Components

Component	Cat. No. E0825-01 25 reactions of 20 µl	Cat. No. E0825-02 100 reactions of 20 µl
2x PRO RT-qPCR SG Buffer	1 x 300 µl	2 x 600 µl
SG PRO Enzyme Mix	25 µl	100 µl
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 0.5 ml	2 x 1 ml

SG OneStep PRO RT-qPCR kit, plus ROX Solution

Kit Components

Component	Cat. No. E0826-01 25 reactions of 20 µl	Cat. No. E0826-02 100 reactions of 20 µl
2x PRO RT-qPCR SG Buffer	1 x 300 µl	2 x 600 µl
ROX Solution, 25 µM	15 µl	60 µl
SG PRO Enzyme Mix	25 µl	100 µl
Thermolabile UNG (uracil-N-glycosylase) 1 U/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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The kit provides:

- 2x PRO RT-qPCR SG Buffer 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. Allows as option to use thermolabile uracil-N-glycosylase (UNG).
- The SG PRO Enzyme Mix contains unique highly sensitive and thermostable reverse transcriptase and DNA Polymerase, RNase Inhibitor and SYBR Green I dye.
- Reverse transcriptase works in a wide temperature range from 52-72°C without losing specificity and sensitivity.
- Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA.
- Proofreading is maintained during both reverse transcription and PCR steps.
- 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 any real-time cycler.
- Kit contains thermolabile uracil-N-glycosylase (UNG) that is optimized for RT-qPCR reactions.
- If cyclers from Applied Biosystems are used ROX passive reference dye is necessary. SG OneStep RT-qPCR 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	0.5 µl	500 nM
Applied Biosystems: 7500, ViiA 7, 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	-

Protocol

Preparation of PCR reaction:

Component	Volume/reaction	Final concentration
2x PRO RT-qPCR SG Buffer	10 µl	1 x
Forward Primer	Variable	0.4 µM
Reverse Primer	Variable	0.4 µM
Template RNA	Variable	≤400 ng
SG PRO Enzyme Mix	1 µl	1 µl/ reaction
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	0.2 µl	0.2 U/reaction
Water, nuclease free	To 20 µl	-
Total volume	20 µl	-

Notes:

1. Minimize thaw-freeze cycles of 2x PRO RT-qPCR SG Buffer, keep SG Enzyme Mix and ROX solution on ice and minimize light exposure during handling to avoid loss of fluorescent signal intensity.
2. Thaw and gently vortex before use 2x PRO RT-qPCR SG Buffer.
3. 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.
4. Optimal amplicon length in real-time RT-PCR using SYBR Green I is 70-150 bp.
5. To avoid amplification from genomic DNA design exon-exon primers.
6. Set up RT-PCR reactions on ice to minimize RNA template degradation.
7. The RNA template (≤400 ng/reaction) should be added to the individual PCR tubes or wells containing the whole reaction mix. Centrifuge briefly before placing into a cycler. Check if there are no bubbles left, if yes, spin again.
8. Place the samples in the cycler and start the program.
9. Reverse transcriptase works in a wide range of temperatures 52-72°C. The recommended starting temperature for reverse transcription is 65°C. For individual experiment temperature might be changed.
10. A final primer concentration of 0.3-0.5 µM is usually optimal, but can be individually optimized in the range of 0.1 µM to 1 µM. The recommended starting concentration is 0.4 µM. Raising primer concentration may increase reverse transcription sensitivity and PCR efficiency, but negatively affect RT-PCR specificity. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
11. Readjust the threshold value for analysis of every run.
12. 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.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Reverse Transcription	65°C	30 min	1
Initial Denaturation	98°C	20 s	1
Denaturation Annealing/Extension/Data acquisition	98°C 60°C	10 s 20 s	40-45
Cooling	4°C	Indefinite	1

Notes:

1. Prior to reverse transcription step at 65°C also thermolabile uracil-N-glycosylase might be used. Do not use UNG from *E.coli*, UNG will degrade all newly synthetized cDNA.
2. Thermolabile UNG is inactivated at temperature above 50°C during RT 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. 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.