MANUFACTURED BY EURx

roboklon

SG 1-Step PRO RT-qPCR Kit

Kit Components:

- The **SG Pro Enzyme Mix** contains a unique, extremely thermostable, highly sensitive reverse transcriptase, DNA Polymerase, RNAse Inhibitor and SYBR Green I dye, respectively.
- **2x PRO RT-qPCR SG Buffer** is a universal reaction buffer with dNTPs (dTTP is partially replaced with dUTP) and 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.** E0825-01 25 reactions, 20 µl each, 500 µl [1x] final volume	**Cat. No.** E0825-02 100 reactions, 20 µl each, 2.0 ml [1x] final volume
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 1.0 ml

SG qPCR Master Mix (2x) plus ROX Solution

Component	**Cat. No.** E0826-01 25 reactions, 20 µl each, 500 µl [1x] final volume	**Cat. No.** E0826-02 100 reactions, 20 µl each, 2.0 ml [1x] final volume
2x PRO RT-qPCR SG Buffer	1 x 300 µl	2 x 600 µl
SG PRO Enzyme Mix	25 µl	100 µl
ROX Solution, 25 µM	15 µl	60 µl
Thermolabile UNG (Uracil-N-Glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 0,5 ml	2 x 1.0 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 and a highly processive hot start Perpetual Taq DNA Polymerase in an easy to use format.

Description:

- 2x PRO RT-qPCR SG Buffer is a universal reaction buffer with dNTPs (dTTP is partially replaced with dUTP for optional UNG-mediated protection from carryover-cross-contamination).
- SG PRO Enzyme Mix contains a unique highly sensitive and thermostable reverse transcriptase, furthermore DNA Polymerase, RNase Inhibitor and SYBR Green I dye.
- The kit contains thermolabile uracil-N-glycosylase (UNG) that is optimized for RT-qPCR reactions.
- SG OneStep PRO RT-qPCR kit is compatible with most commercially available real-time PCR cyclers.
- The reverse transcriptase works in a broad 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.
- If cyclers from Applied Biosystems are used, the usage of ROX as a passive reference dye is mandatory. SG OneStep RT-qPCR kit is provided in two variants: without ROX and with ROX Solution provided in a separate tube. The table below shows recommended amount of ROX (25 µM) required for commonly used PCR cycler models.

MANUFACTURED BY EURx

roboklon

SG 1-Step PRO RT-qPCR Kit

REAL TIME RT-qPCR PROTOCOL (1)

qPCR- Protocol

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

Instrument	Amount of ROX per 20 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.4 µl	500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.4 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 PRO RT-qPCR SG Buffer	10 µl	1 x
Forward Primer	Variable	0.4 µM
Reverse Primer	Variable	0,4 µM
Template RNA	Variable	less than 400 ng
Optional: ROX Solution, 25 µM	0.4 µl or 0.4 µl 10 x diluted	500 nM 50 nM
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	0.2 µl	0.2 U / reaction
SG PRO Enzyme Mix	1 µl	1 µl / reaction
Water, nuclease free	To 20 µl	-
Total volume	20 µl	-

Notes:

1. **Minimize freeze-thaw cycles** of 2x PRO RT-qPCR SG Buffer. Always keep SG 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 PRO RT-qPCR SG Buffer before use.
3. **Reaction Volume.** A reaction volume of 20 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 (400 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 52°C and 72°C. The recommended starting temperature for reverse transcription is 65°C. For individual experimental requirements, the RT incubation temperature temperature might be changed.
10. **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.
11. **Readjust the threshold value** for analysis of every run.
12. **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 PRO RT-qPCR Kit

REALTIME RT-qPCR PROTOCOL (2)

qPCR- Protocol - Thermal Cycling Conditions

Thermal Cycling Conditions:

Step	Tempera-ture	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	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 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.
2. **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.
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. Melting temperatures of the specific product and primer-dimers may overlap.