EURx® MOLECULAR BIOLOGY PRODUCTS

smART RT-qPCR Kit

Cat. No.	size
E0806-01	25 reactions
E0806-02	100 reactions

smART Unit Definition:

One unit incorporates 1 nmol of TTP into acid precipitable material in 10 min at 37°C using poly(A): oligo dT as a template: primer (1).

Storage Conditions: Store at -20°C.

Concentration: 200 U/µl

Quality Control:

All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single- and double-stranded DNase activities.

References:

1. *Houts, G.E., Masakau, M., Ellis, C., Beard, D. and Beard,J.W. (1979) J. Virol. 29, 517-522.*

smART RT-qPCR Kit is a complete system for efficient synthesis of first strand cDNA and qPCR. Kit contains smART Reverse Transcriptase with increased thermostability and processivity. The real time qPCR is performed with easy to use SG qPCR Master Mix (2x).

Description:

- Kit based on processive smART Reverse Transcriptase.
- Increased thermal stability of reverse transcription in the range of 37°C to 65°C.
- Downstream application: qPCR with SYBR Green I in optimized Master Mix (2x) with dATP, dCTP, dGTP, dTTP/dUTP.
- Contains highly efficient Hot Start DNA Polymerase ideal for real time diagnostic.
- Kit contains UNG (uracil-N-glycosylase) to prevent cross contamination.

COMPONENT:	E0806-01	E0806-02
smART (200 U/µl)	25 µl	100 µl
5 x cDNA Buffer	100 µl	400 µl
0.1 M DTT	50 µl	200 µl
Oligo $(dT)_{20}$ (50 µM)	25 µl	100 µl
Random hexamers (200 ng/µl)	25 µl	100 µl
10 mM dNTPs Mix	25 µl	100 µl
RNase Inhibitor (50 U/µl)	12.5 µl	50 µl
SG qPCR Master Mix (2x)	350 µl	1.4 ml
UNG (uracil-N-glycosylase) (1 U/µl)	6.25 µl	25 µl
RNase-free Water	1 ml	4 x 1 ml

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

I First strand cDNA synthesis:

1. Place 5 x cDNA Buffer at room temperature, thaw and vortex gently.
2. Assambly reaction in RNase-free tube as follows:

Component:	Amount:
RNA (10 ng-5 µg)	x µl
primer*	1 µl
10 mM dNTP Mix	1 µl
RNase-free Water	to 12.5 µl

*50 µM Oligo(dT)$_{20}$, 200 ng/µl random hexamer primer or 10 µM reverse gene specific primer.

3. *Optional*. If GC-rich or structured RNA template is used, mix gently, centrifuge briefly and incubate 5 min at 65°C then chill on ice.
4. Add the following components to the reaction tube in the indicated order:

Component:	Amount:
5 x cDNA Buffer	4 µl
DTT 0.1 M	2 µl
RNase Inhibitor 50 U/µl	0.5 µl
smART (200 U/µl)	1 µl
Total volume	20 µl

5. Transfer the sample to preheated to appropriate temperature thermal cycler. Incubate as follows:

Oligo(dT)$_{20}$ primed:	30-60 min at 50°C (or 37-65°C)
Gene specific primed:	30-60 min at 50°C (or 37-65°C)
Random hexamer primed:	25°C for 10 min, followed by 20-50 min at 50°C (or 37-65°C).

NOTE

50°C is suitable temperature for most targets. For G-C rich RNA templates or with complex secondary structure temperature can be increased to 65°C.

6. Terminate the reaction by incubating at 85°C for 5 min.
7. cDNA is ready for PCR, can be used immediately or stored at -20°C. Use 2-5 µl for 50 µl PCR or qPCR.

II qPCR with SG qPCR Master Mix (2x)

Store SG qPCR Master Mix (2x) at 4°C in the dark. The performance of the master mix is guaranteed up to 9 months. Freezing and thawing of the master mix should be avoided.

1. Mix as follows all reagents in 0.2-0.5 ml tube.

Component	Volume/reaction	Final concentration
SG qPCR Master Mix (2x)	12.5 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.3—0.5 µM
Reverse Primer	Variable	0.3—0.5 µM
Template DNA	Variable	≤ 3 µl
Optional: 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 exposure of SG qPCR Master Mix (2x) to light during handling to avoid loss of fluorescent signal intensity.
2. 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.
3. Optimal amplicon length in real-time PCR using SYBR Green I is 70-200 bp.
4. Thaw if needed, gently vortex and briefly centrifuge all solutions.
5. Set up PCR reactions at room temperature. Use of SG qPCR Master Mix (2x) allows room temperature reaction setup.
6. Prepare a reaction master mix by adding all the reaction components except template cDNA.
7. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
8. Add template cDNA to the individual PCR tubes or wells containing the reaction mix. For two-step RT-PCR, the volume of cDNA added should not exceed 10% of the final PCR volume.
9. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
10. Place the samples in the cycler and start the program.
11. Standard concentration of $MgCl_2$ in real-time PCR reaction is 2.5 mM (as provided with the 1 x SG qPCR Master Mix). 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 a reaction.
12. 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 0.5 µM. Raising primer concentration may increase PCR efficiency, but negatively affect PCR specificity. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
13. Readjust the threshold value for analysis of every run.
14. 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. Factors are used to compensate for any excitation or pipetting variations.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Optional: UNG pre-treatment	50°C	2 min	1
Initial Denaturation	95°C	15 min	1
Denaturation	94°C	15 s	35-45
Annealing	50–60°C	30 s	
Extension	72°C	30 s	
Optional: Data acquisition	X°C	15 s	
Cooling	4°C	Indefinite	1

Notes:

1. The incubation step of 50°C for 2 minutes must be added if a uracil-N-glycosylase is used to prevent carryover contamination. UNG degrades any dUMP-containing PCR products.
2. 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 in PCR reaction the duration of the initial denaturation step can be reduced to 2-5 min at 95°C.
3. UNG activity may be partially restored at temperatures lower than 55°C due to refolding. It is recommended to perform PCR using a temperature equal 55°C or above for the annealing step. After completing the PCR cool reactions to 4°C and load directly on a gel or store frozen.
4. 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.
5. 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.
6. 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.