EURx® MOLECULAR BIOLOGY PRODUCTS

smART First Strand cDNA Synthesis Kit

Cat. No.	size
E0804-01	2 000 U (10 reactions)
E0804-02	10 000 U (50 reactions)
E0804-03	4 x 10 000 U (200 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 First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA. Kit consists of smART Reverse Transcriptase with reduced RNase H activity, increased thermostability and processivity. smART Reverse Transcriptase acts as a DNA polymerase that uses single-stranded RNA, DNA, or an RNA-DNA hybrid (using a primer) to synthesize a complementary DNA strand. smART is used for the preparation of cDNA libraries or for first strand cDNA synthesis for use in RT-PCR and RT-qPCR reactions.

Description:

- Reduced RNase H activity.
- Increased thermal stability in the range of 37°C to 65°C.
- Downstream application: PCR and qPCR.
- Ideal for cloning and diagnostic purposes targets up to 7kb.

COMPONENT:	E0804-01	E0804-02
smART (200 U/µl)	10 µl	50 µl
5 x cDNA Buffer	100 µl	500 µl
0.1 M DTT	50 µl	250 µl
Oligo $(dT)_{20}$ (50 µM)	10 µl	50 µl
Random hexamers (200 ng/µl)	10 µl	50 µl
10 mM dNTPs Mix	10 µl	50 µl
RNase Inhibitor (50 U/µl)	5 µl	25 µl
RNase-free Water	200 µl	1 ml

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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 PCR

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. Normally, 2 µl of cDNA is used as template for subsequent PCR or qPCR in 25 µl total volume.
cDNA is compatible with various EURx PCR amplification products.
For standard PCRs we recommend to use OptiTaq DNA Polymerase Cat. No. E2600 or depending on the downstream application tiOptiTaq DNA Polymerase Cat. No. 2725 or Perpetual OptiTaq DNA Polymerase Cat. No. E2720.
Regarding qPCRs cDNA is optimized for SYBR Green I dye (product Cat. No.: E0401, E0402, E0411, E0412) or for Probe detection (product Cat. No.: E0420, E0421, E0422, E0423).

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